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PHYSIOLOGICAL REVIEWS

VOLUME II

BALTIMORE, MD.
1922

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Edited for

THE AMERICAN PHYSIOLOGICAL SOCIETY

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Vol. II, No. 1

Issued January, 1922

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the Act of March 8, 1879

Made in United States of America

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Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
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PHYSIOLOGICAL REVIEWS

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WAVERLY PRESS
The Williams & Wilkins Company

PHYSIOLOGICAL REVIEWS

VOL. II JANUARY, 1922 No. 1

TRANSMISSION OF PHYSIOLOGICAL INFLUENCE IN PROTOPLASMIC SYSTEMS, ESPECIALLY NERVE

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The general problem of the conditions determining the transmission of physiological influence and control in organisms is one of fundamental interest, since it is evident that the living system can develop, function and react as a whole, *i.e.*, in the unified or integrated manner essential to survival, only in so far as physiological processes in any one region occur in correlation with, or in adjustment to, processes in other regions or in the external environment. Each cell, and in a larger sense each individual organism, constitutes a single self-regulating system, the various separate activities of which mutually influence and control one another; normally the metabolic, formative and functional processes are so coördinated as to secure a definite and constant unity of structure and activity in the whole organism. This unity is apparent at all stages of development, although it exhibits itself in its most striking form in the integration of function and response characteristic of the free-living adult. Obviously any such functional integration implies a ready transmission of influence between the different parts of the living system. In multicellular organisms we now recognize two qualitatively distinct types of such transmission (1). The first depends upon the transport of definite chemical substances from region to region, usually in the circulation; those substances, through their special influence on metabolism or physiological activity, control processes at a distance from their origin (hormone influence; influence of the transportative type (2)). But in addition to this directly chemical type of distance-effect there is a second, evidently of a more fundamental kind, consisting in the rapid transmission of excitatory and inhibitory influence by a process which is independent of direct material transfer; this process is concerned in the majority of responses to stimulation and attains its highest development in nervous transmission. Motor and sensory reactions, where promptness of response is essential, are especially subject to this type of control. The term "protoplasmic transmission" (3) seems a satisfactory designation, since continuity of living protoplasm—or at least close contact between the protoplasmic structures intervening between the stimulated and the responding areas—seems necessary. Evidently some physiological change in the living protoplasm forms the vehicle of such transmissions.

It is with this second type of transmission that the present article is more immediately concerned. In general, living "irritable" protoplasm is so constituted that the physiological effects produced by local alteration are transmitted more or less rapidly to other regions. All such effects must have a chemical basis. In seeking for inorganic analogies, therefore, a natural comparison is with the "chemical distance-action" (4) well known in polyphasic systems of a certain well-defined type of constitution. Protoplasm represents a reaction-system in which the reacting materials are chiefly in aqueous solution. It is well known that a chemical reaction in one region of a solution may influence another reaction at a distant region of the same solution, without any material transfer between the two regions, provided both reactions occur at the surface of some electrically conducting phase (*e.g.*, metal) which forms with the solution a complete electrical circuit. In any battery the two electrodes together with the connecting wire represent such a conducting phase; in this case the rate of chemical action at the anode is controlled by that at the cathode in the definite manner prescribed by Faraday's law. In general, in any system having the essential constitution of a battery, *viz.*, metal (or other continuous conducting phase) in contact with an electrolyte solution, and with some asymmetry of composition or concentration at two regions of the contact-surface, chemical influence is transmitted instantaneously between the two regions. The essential condition for this transmission is the flow of electricity around the circuit. The electric current can pass between solution and electrode only in association with chemical reactions, which are dependent on the transfer of electrons between the two phases. Since the direction of this electron transfer, relatively to the solution, differs at the two electrode areas, the reactions there occurring exhibit, in addition to the interdependence just defined, a *reciprocality* in their general chemical character; *i.e.*, oxidation at the anode is associated with reduction at the cathode. These considerations also explain why the reactions are confined to the layer of molecules adjoining the interface, and do not occur in the body of the solution or of the metal.

The protoplasmic transmissions are examples of a process which may be called "physiological distance-action," and in fact various significant analogies with chemical distance-action are apparent, *e.g.*, rapidity of transmission, frequent reciprocality of effect in the two regions influencing each other, susceptibility to electrical influence. Such resemblances suggest that the essential determining conditions in the chemical and in the physiological forms of distance-action are identical and depend on certain fundamental similarities of physico-chemical constitution in the two types of system under comparison.

What we observe in living organisms is that variations of physiological activity in certain regions—implying variations in the rate or character of the underlying chemical or metabolic processess—influence other physiological processes occurring at a distance from the active region. In some cases this influence is of an exciting or initiatory kind (stimulation), in others repressive or inhibitory (inhibition). It may have reference to the most fundamental vital processes, such as growth, or to special activities of the most varied nature. Illustrations of these different effects of transmitted influence are found in all higher animals and plants, and detailed enumeration is unnecessary.

A fundamental feature of this transmissive influence is the reciprocality of action which it often shows in the two regions mutually affected. Acceleration of a physiological process in one region is frequently associated with its retardation or prevention in regions adjoining. This effect is seen with especial clearness in growth-processes. Rapidly growing organs or parts of animals and plants often exert an inhibitory influence on the growth of neighboring parts. A well known instance is the influence of the apical buds of seedlings or of growing branches in repressing the growth of the cotyledonary or axillary buds in adjoining regions. If the apical buds are removed, or if their growth is repressed by cold or anesthetization, the previously inhibited lateral buds sprout out (5). The growth-inhibiting influence thus rendered evident cannot be due to the transport of inhibitory substances from the actively growing zone, as once supposed, since it may be prevented from acting by conditions (cold (6), anesthetization (5), heat-injury (7)) which do not interfere with the flow of materials along the stem. In animals a similar repressive influence is exerted by actively growing or developing or functioning regions; this is best illustrated by the phenomena of regeneration, in which the removal of a part initiates growth and differentiation in regions which formerly were quiescent. Such regions thus show themselves capable of independent growth and differentiation; but apparently in the intact organism these processes are held in check by the influence of the metabolically more active or "dominant" regions adjoining; when removed from this influence—or "physiologically isolated," to use Child's term (8)—they proceed to grow and develop on their own account. In Child's recent books (2), (8) these phenomena of inhibition and dominance in the growth of animals are discussed in detail. Further illustrations of these effects as seen in the growth of vertebrate embryos are to be found in the recent experimental studies of Bellamy (9) and Stockard (10). In the Fundulus egg the formation of one actively growing embryonic axis in the blastodisc normally prevents the development of another, so that only a single embryo is formed from each egg; but under certain abnormal conditions (*e.g.*, cold) two such embryonic axes may begin growth; if these are equal in vigor, twinning may result; but if one happens to exceed the other in this respect, it exerts upon the latter an inhibitory influence, with failure of development or regression as a result (10).

According to Stockard (10), the normal sequence of growth and development in the different parts of the embryo is determined largely by influences of this kind. Active proliferation in one embryonic area prevents this process in other areas; when the period of most active growth in one region is passed, another region is released from this restraint and passes through its own cycle of rapid growth, and so on in turn. In Loeb's extensive studies of the growth and regeneration of the plant Bryophyllum from detached leaves and stems (11) he describes how the growth of buds on a piece of stem inhibits the growth of roots from a leaf attached to the same stem; he cites various instances of reciprocal influence between the growth of different organs and in general concludes: "if an organ *a* inhibits the regeneration or growth of an organ *b*, the organ *b* often accelerates and favors the regeneration in *a*" (11; *cf.* 1915, p. 276). Such reciprocality is seen not only in the phenomena of growth, but is especially evident in the normal functioning of the central nervous system in higher animals, where the activity of any motor group of neurons represses that of the antagonist group (1). This normal or physiological type of reciprocal influence has an interesting correspondence with the reciprocality exhibited in the effects produced at anode and cathode when a constant current is passed through an irritable living tissue or organism (polar stimulation and inhibition, electrotonus, galvanotropism), and in all probability has the same essential basis.

Examples of the transmission of inhibitory or excitatory influence to a distance without evidence of reciprocal effects are also frequent. In many animals, both vertebrate and invertebrate, impulses entering the heart through certain nerves (cardio-inhibitory) check or arrest the action of this organ. The propagation of excitatory influence is a more frequently observed phenomenon. In plants Darwin first showed that stimulation of the root-tip, by light or otherwise, modified growth higher up the stem, and many similar instances are now known; and in all animals from protozoa to vertebrata certain forms of local stimulation are observed to initiate, accelerate or modify physiological processes (motor, secretory, etc.) at a distance from the region directly stimulated. In such cases the functional or responding (effector) region is connected with the stimulated (or receptor) region by protoplasmic tracts, differentiated as nerves in higher animals; these conduct or transmit the excitatory influence in the form of waves of chemical and physical alteration travelling at moderate velocities. These velocities are highly variable in different organisms and tissues, ranging from a few centimeters per second or less to maxima of about 100 meters per second in the motor nerves of warm-blooded vertebrates.[1]

Transmissions of this type form the chief subject of consideration in the present article. The essential problem has reference to the general physico-chemical nature of such transmissions, as they occur in all irritable and conductive forms of protoplasm, rather than to the special conditions prevailing in single tissues like nerve. The irritable living substance, wherever found, must have such a constitution that transmission of chemical or metabolic influence to a distance is a constant feature of its activity. Hence the problem of the essential nature of protoplasmic structure or organization is intimately related to the problem of protoplasmic transmission. What kind of structure must any reaction-system have in order to be able to transmit chemical effects to a distance in the above manner? This question leads to a consideration of inorganic systems which exhibit similar powers of transmission, and to a comparison of such systems with living systems, with a view to ascertaining what fundamental features of structure or composition the two types have in common.

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[1] In the motor nerves of different animals there is a direct correlation between the velocity of transmission in a nerve and the rate of contraction of the muscle which it innervates (12). Data on velocities are given in many textbooks. *Cf.* Piper (13) for the special conditions in mammalian nerve, Keith Lucas (14) for frog's nerve.

A simple example of physico-chemical distance-action which bears many suggestive resemblances to the reciprocally acting type of physiological distance-action is seen in the formation of precipitation-structures from metals immersed in solutions of salts the anions of which form insoluble salts with the metal (ferro- and ferricyanides, carbonates, hydrates, etc.) (15). These effects are well shown in a combination of the two metals, iron and zinc, in an appropriate solution of potassium ferricyanide. When a strip of zinc foil and a small length of iron wire are placed separately in a watch glass containing this solution (4 per cent K_3FeCy_6 *plus* 0.5 per cent NaCl, dissolved in dilute egg-white or gelatine to furnish a protective colloid), each metal reacts characteristically. A precipitate of ferrous ferricyanide is rapidly formed from the surface of the iron; this precipitate is deposited chiefly in the form of slender blue-green filaments and tubules which elongate rapidly and soon assume a highly characteristic hypha-like appearance; within a few minutes the wire is covered with a dense growth of these precipitation-filaments, some of which may grow later to a length of several centimeters. The zinc reacts with the solution more slowly, and even after some hours usually shows only a few scattered vesicles or filaments of zinc ferricyanide. If, however, the metals are allowed to react while in contact with each other, *e.g.*, with a strip of zinc an inch long attached at one end to an iron wire of similar length, the reaction of both metals is altered in a striking manner. The formation of filaments from the iron is completely prevented, while their formation from the zinc is markedly accelerated; within an hour the zinc is covered with a characteristic growth of vesicular and tubular precipitation-structures, while the iron remains bright and bare as at first. If now the iron wire is severed with a pair of scissors, the detached piece instantly begins to form filaments, while the still attached part remains unchanged. From this simple experiment it appears that the reaction of the iron is prevented only while it is in metallic connection with the zinc; one might express the facts—using physiological language—by saying that the zinc "inhibits" the growth of filaments from the iron, while the iron "promotes" growth from the zinc. Each metal thus exerts through its contact an influence which modifies the chemical processes at the surface of the other metal, and in a characteristically reciprocal manner. This influence is well marked for a distance of several centimeters from the metallic junction; its intensity decreases progressively with increase in the distance from the contact, and beyond a certain distance the effect becomes inappreciable. Such a gradient of chemical influence is comparable with the gradient of physiological influence or growth-inhibiting "dominance" seen in developing or regenerating planarians and other animals (2), (6), (16).

The explanation of these chemical transmissions or distance-effects in combinations of metals is simple. Zinc and iron, in contact with each other and with the electrolyte solution, form an electrical couple in which, because of its higher electrolytic solution-tension, the zinc is anode, while the iron is cathode. The direction of flow of the electric current (positive stream) is thus from zinc to solution and from solution to iron; hence the passage of the zinc ions into solution is promoted and that of the iron ions is hindered; this is especially the case where the current intensity is greatest, near the junction of the two metals. Any metal with a lower solution-tension than zinc (Cu, Ag, Pt, Pb, Hg, etc.) resembles iron in its power of promoting precipitate-formation from zinc; conversely the contact of metals of higher solution-tension than iron (Zn, Mn, Mg, Cd, etc.) inhibits the formation of filaments from the latter metal. The precipitate is simply an indicator of the passage of the metallic ions into solution; incidentally it is built up to form a definite type of filamentous or vesicular structure characteristic for each metal. The rate of the reaction and the distance through which the chemical influence is perceptible depend essentially on two factors, (*a*) the potential-difference between the two metals, and (*b*) the electrical resistance of the solution. Obviously the intensity of the current passing between metal and solution at any point on the surface of either metal must fall off progressively with increase in the distance from the contact of the two metals, since the electrical resistance of the circuit which includes the point considered depends chiefly on the length of the column of solution between that point and the metallic junction. Hence near the junction the mutual influence of the two metals is greatest, and beyond a certain distance it ceases to be perceptible. Both the transmission of the chemical influence to a distance and the existence of a gradient of such influence are thus directly dependent on the electrical nature of the chief factors.

It appears probable that certain forms of physiological distance-action, especially the growth-inhibiting and other reciprocally acting influences cited above, are to be referred to general conditions of the kind just described. Electrical potential-differences have long been known to exist between actively growing regions and the less active regions (17); hence currents must flow in definite directions through the growing organism (18); and it is also known from the facts of galvanotropism that electric currents influence growth. According to the present hypothesis, those cases where there exists a well-defined gradient of growth-determining influence fall in the general category of effects illustrating the influence of the electric current on growth. Already there is much evidence indicating that in general actively growing or regenerating regions of an organism are negative (like physiologically active regions in other tissues) relatively to quiescent regions (19), (20), (21); *i.e.*, the direction of the bioelectric "growth currents" (positive stream) is from medium to living protoplasm in the growing regions and from protoplasm to medium in the adjoining quiescent regions. In these two regions the currents have opposite directions relatively to the cell-surfaces; and to this difference of direction must correspond a difference in the influence on growth, since the constant current always exhibits polarity in its physiological action. This is presumably the basis of the reciprocality described above. Whether the conditions of local current-intensity, resistance, and susceptibility to weak currents correspond to the requirements of the above theoretical conceptions in all details can be determined only by future experiment. At the present time further and more exact work on the effects of the electric current on growth is much to be desired.[1a]

Transmissions by means of the direct influence of bioelectric currents, exerted under conditions similar to those of the above metallic models, *i.e.*, where the intensity of the transmitted effect necessarily decreases with distance from the physiologically active or dominant region. cannot, however, form the basis of the rapid forms of excitation and transmission exhibited in nervous and muscular action. In nerve especially the transmitted influence shows no decrease in intensity as the distance from its origin increases; this is shown clearly by the case of "trapped waves" in rings of medusa tissue (22). A self-propagating wave of excitation, associated with a local bioelectric circuit, passes along the conducting tissue and arouses physiological activity at all points along its path and in the terminal organ (phenomena of conduction and innervation). Yet here again simple types of inorganic process are known which simulate this second form of protoplasmic transmission in many of its most characteristic details.

[1a] While the present review is in proof, an experimental study by Lund (99) on the electrical control of regeneration in the cut stems of the hydroid Obelia is announced as about to appear in the Journal of Experimental Zoölogy. The formation of new hydranths is promoted where the positive stream of a weak current enters the cut end of the stem, and inhibited where it leaves. Sven Ingvar (100) has recently obtained polar directive effects of a similar kind in studying the influence of weak constant currents on the outgrowth of the processes in embryonic nerve-cells.

Nervous transmission is the physiological type-phenomenon of this class. In all such phenomena the essential effect is the spreading of a certain active state (23); this state is initiated by some local change caused usually by some external agent or "stimulus". We may thus distinguish between (*a*) the local effect produced by the stimulating agent at its point of application and (*b*) the physical or chemical disturbance there set up which is propagated to a distance (conducted effect) (24). This "propagated disturbance"[2] (activation-wave, nerve-impulse, etc.) has the property of altering physiological activity wherever it extends, *e.g.*, of stimulating or inhibiting activity in the terminal organ. The essential problems therefore are: why it should be propagated, or spread from region to region along the conducting tissue, and why it should initiate or alter physiological activity in the same tissue or in another tissue (*e.g.*, muscle) to which it is transmitted.

There are also forms of transmission—apparently intermediate between the two types above distinguished—in which a conducted influence is transmitted through an often considerable distance, but with a progressive decrease in "intensity" or physiological effectiveness as it travels. This is the case of "conduction with decrement" (25). Certain normal types of physiological gradient seem to depend on transmission of this kind, as indicated by the fact that the influence may be intercepted by cold or anesthetization (5), (6). In the central nervous system it appears to play an important part (14); thus the penetration of the impulses determining sensory impressions, association processes, or reflexes in the brain or spinal cord is limited and varies with the physiological state (sleep, fatigue, etc.) This decrement type of conduction can be artifically induced in nerve or muscle by cold, fatigue or anesthesia. For example, an impulse initiated in a normal region of a nerve penetrates at a lessened velocity for a certain distance along an anesthetized stretch, but is eventually extinguished if the latter is of sufficient length; if, however, the impulse succeeds in traversing this "region of decrement" and emerges into the normal region beyond, it there regains its original velocity, amplitude and other properties (26) (see below, p. 30). If "intensity" is measured by the ability to penetrate a region of known decrement, as Lucas has proposed (14), it is evident that this property progressively decreases as the impulse travels along such a region.

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[2] Keith Lucas' term; for the experimental basis of the distinction between local and propagated effects *cf.* Lucas and Adrian (24a).

In any transmission without decrement, such as we find in a normal motor nerve, the process occurring at any stimulated region of the transmitting element initiates in adjoining regions a process which is similar qualitatively and quantitatively to that at the original region; and each region thus secondarily activated influences the region beyond in the same manner. In a uniformly constituted elongated element of this type propagation for an indefinite distance at a uniform speed is to be expected; this corresponds to what we observe in nerve. Conditions of an analogous kind are seen in the transmission of a wave of combustion along a fuse; at any instant there exists for a certain distance in advance of the burning region a certain gradient of temperature; within a portion of this gradient the temperature is above the ignition-temperature; and the speed of travel depends on this critical distance and on the rate at which the heat-generating reaction proceeds (*i.e.*, on the local reaction-velocity). In the case of the conducting protoplasmic element the transmission from the active to the adjoining resting region is apparently a result not of the establishment of a difference of *temperature*, but of a difference of *electrical potential*. In other respects, however, the analogy is an instructive one. According to the present conception, up to a certain distance in advance of the active region (R_1, fig. 1) the current of the local active-resting circuit is sufficiently intense to cause secondary electrical excitation; the length, s, of this critical distance, and the rate, v, at which the variation of potential rises to its maximum in the active region (a measure of the local reaction-velocity) determine the speed of propagation, P. Expressed algebraically, $P = Ksv$, or $P = Ks/t$, K being a proportionality-factor and t the time occupied by the variation of potential (27). There exists in fact a close proportionality between the rate at which the bio-electric variation rises to its maximum in different conducting tissues and the rate of transmission of the excitation-wave (28).

Inorganic systems exhibiting this form of transmission, *i.e.*, transmission by means of the electrochemical effects produced near the boundary between altered and unaltered regions, are well known, and usually consist of oxidizable metals immersed in electrolyte solutions containing an oxidizing agent which acts energetically enough to form a continuous film of oxidation-product over the whole surface of the metal. Mercury in hydrogen peroxide (29) and passive metals, especially iron, in strongly oxidizing solutions like nitric acid are the best known examples (30). The spreading action depends upon the formation of local circuits between the film-covered and the free metallic surfaces; through the electrolytic action of these circuits the film is locally removed; and this effect is automatically self-propagating, since wherever film-covered and free metallic surfaces adjoin each other a similar circuit is formed and the effect is repeated. That phenomena of this type exhibit in many cases remarkably close analogies to physiological processes (in rhythm, automaticity, conduction, etc.) has been recognized by a number of investigators. Bredig and his students have investigated the conditions in the Hg-H_2O_2 system (31), although without special reference to the problem of protoplasmic transmission; and the transmission of the activation-wave over the surface of passive iron in nitric acid—a phenomenon with especially striking physiological analogies—has been compared to nervous transmission by Ostwald (32), Heathcote (32) and others (33).

Recently I have studied in some detail (3), (33) the analogies of this phenomenon to protoplasmic transmission of the nervous type, and have endeavored to account for the above similarities on the basis of the fundamental features of structure and activity possessed in common by all polyphasic systems having thin chemically alterable interfacial films (34). The presence of films deposited at the interphase surfaces determines the properties of many such systems, *e.g.*, the stability and physical consistency of emulsions and certain types of gel.[3] Local variations in the chemical composition, permeability or other properties of these films should theoretically give rise to potential differences between adjacent areas; and under certain conditions it is conceivable that local electric currents may thus arise which secondarily may influence chemical processes in the system. Since living protoplasm is in fact a film-partitioned system, and since many irritable cells are known to be bounded from their media by semi-permeable membranes whose composition and properties vary with the physiological state of the cell, it is perhaps not surprising that protoplasmic systems should exhibit transmission-phenomena of a type closely similar to those found in film-covered metallic systems such as passive iron in nitric acid.

The phenomena of passivity in metals are well known (30), and only a brief account of the more relevant facts need be given here. An iron wire dipped in strong nitric acid or other suitable oxidising agent and then transferred to weaker acid (sp. gr. 1.20, in which the normal or

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[3] For the structure and properties of emulsions and related systems *cf.* the very full review of W. D. Bancroft, *The Theory of Emulsification* (35).

"active" metal dissolves rapidly) shows no reaction; the metal is in the so called "passive" state, but it may be made to react (or "activated") by touching with ordinary active iron or other base metal, or by mechanical means (bending, scraping with glass, etc.). The reaction, which is accompanied by effervescence and the production of dark-colored lower oxide, then sweeps over the whole surface of the metal, at a rate which varies with the conditions but may attain some hundred centimeters per second, a velocity of the same order as rapid protoplasmic transmissions. Of especial interest from the physiological standpoint is the fact that in HNO_3 of more than a certain concentration (sp. gr. *ca.* 1.24) the reaction is temporary, the metal reverting spontaneously to the passive state; after an interval—more or less prolonged according to the concentration of acid—during which reactivation is difficult or partial (because of imperfect transmission) the metal can be reactivated as before (37).

It is now generally agreed that passivity is due to the presence of a thin, coherent and impermeable surface-layer of oxidation-product (probably higher oxide), and that during activation this layer is removed by electrochemical reduction near the boundary between the active and the passive regions. Any region of a passive wire may be made locally active by removing or interrupting the passivating film, mechanically, chemically or otherwise. Such an active region represents the anode of the local couple thus formed; at the adjoining still passive or cathodal area the surface-film is reduced and disintegrated; this region then becomes active in its turn, *i.e.*, anodal, and the same sequence of effects is repeated at the regions beyond. Hence a wave-like spread of activity over the whole surface follows any sufficient local alteration. Wherever the surface-film is removed, interrupted or rendered permeable, the metal at once reacts with the acid. In repassivation the film is restored and the reaction ceases. The reason why this repassivating reaction occurs automatically in strong HNO_3 is that each region as it becomes active becomes also anodal, and is hence subjected to the anodal oxidizing influence, in addition to that exercised by the HNO_3 itself. In general, the variations in the reaction of the metal with the acid thus depend on variations in the film-structure of the system (33). Under certain conditions these variations are rhythmical, and the film undergoes regular and automatic alternate formation and dissolution, giving rise to a rhythm of reaction resembling in its time-relations and other features physiological rhythms like that of the heart-beat. A similar rhythm is shown by mercury in H_2O_2 (31).

The essential features exhibited in common by transmission in passive iron and in nerve (or similar protoplasmic systems) may be summarized as follows. *1*, A local or initiatory process may be distinguished from a propagated disturbance or wave of reaction. Local alterations of various kinds, mechanical, chemical or electrical, may initiate an activation-wave in either system; once initiated its propagation is automatic and determined by the state of the transmitting wire or filament and of the adjoining solution. The "all or none" character of the reaction in readily transmitting metallic or protoplasmic systems is thus explained. *2*, The local reaction accompanying the passage of the reaction-wave is automatically reversible, but there is always a certain delay (called "refractory period" in the living system) before the system returns to its original irritable and conductive state. (In the passive metal this interval is that required for the formation of a surface-film with the original properties (33).) *3*, In a uniform nerve fiber or passive iron wire the activation-wave shows the same characters at all points along its path. It is associated with a variation of electrical potential, and with a temporary loss of continuity or breakdown (increase of permeability) in a surface-film of chemically alterable material. This latter change is demonstrable in passive iron and in certain slowly conducting protoplasmic systems (*e.g.*, echinoderm egg-cells during the fertilization-reaction (36)), but in nerve the evidence for its existence is indirect. *4*, The propagation-velocities in both systems are of the same order (ranging from a few centimeters to some meters per second), and exhibit a high temperature coefficient (of the order of $Q_{10} = 2$). *5*, In both systems the activation-wave may be retarded or blocked by certain forms of chemical or electrical influence, *e.g.*, electrical polarization (anelectrotonus in the living system, anodal polarization in the passive iron system (37, p. 136)) or chemical alteration (*e.g.*, narcosis in the living system). *6*, Interruption of metallic or protoplasmic continuity prevents the passage of the wave, but activation may be transmitted by contact. This is shown in the living system by certain structural features in the arrangements of neurones in the central nervous system (synapses, end-feet), by the characters of the motor end-plates and other nerve endings, and by the facts of secondary stimulation by bioelectric currents (rheoscopic nerve-muscle preparation, etc.).

The conditions under which a local alteration ("stimulus" in the living system) initiates the activation-wave also show many close parallels in the two systems.[4] The chief of these are as follows: *1*,

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[4] For the condition in the passive iron model *cf.* Science, 1918 (33).

A certain intensity or degree of local alteration is required ("threshold"); too slight a mechanical or electrical disturbance will not start a wave of activation, although it may produce a temporary local effect,—shown by temporary change of potential in the wire, or by the fact that in both cases a sufficiently rapid succession of such subminimal effects will activate (37, p. 134). *2,* A succession of local mechanical or electrical "stimuli", each of which singly is inadequate, will start an activation-wave if the interval between the stimuli is sufficiently brief (summation effect).[5] *3,* Activation by the electric current is a characteristically "polar" effect; the passive wire is activated when it is made cathode in a circuit, but not when it is made anode; in the latter case activation by another agent (*e.g.*, mechanical) is rendered more difficult during the flow of the current (37, p. 136). In the living system the parallels are polar stimulation and polar inhibition (or anelectrotonus). *4,* Too weak an electric current will not activate, no matter how long it is continued (critical or threshold intensity), and a current of sufficient intensity for activation must flow for more than a certain time in order to produce its effect (39). *5,* A current rising too gradually from subminimal to a sufficient intensity will not cause activation; *i.e.*, more than a certain critical rate of change is required in the activating current (40).

Conditions 2, 4 and 5 show that both types of system have an automatic tendency to preserve a certain state—corresponding to the passive or resting state—and to resist displacement from this equilibrium; the disturbing or activating condition must therefore act for a sufficient time and more rapidly than the automatic passivating counter-changes in order to bring the local alteration to the level required to initiate an activation-wave.[6]

Evidently the local alteration or stimulus originates a condition or process which is automatically self-propagating. The originating external agency, *e.g.*, a mechanical impact, produces at its point of application some surface-alteration which is (or may be) quite different from the propagated reaction which follows; its immediate effect, however, appears in all cases to be the establishment of a certain critical potential-difference between the region thus altered and the unaltered region adjoining. In the metallic model the existence of this effect may be shown experimentally by activating mechanically a passive iron wire which is immersed in HNO_3 and connected through a galvanometer with an indifferent electrode (*e.g.*, platinum wire) immersed in the same solution. An ineffective scrape with a glass slide causes a slight temporary excursion of the instrument, indicating a quickly reversed change of potential, while an effective scrape or succession of scrapes causes a larger excursion which is at once followed by that accompanying the full propagated reaction (37, p. 134). In the living system something analogous seems to occur; apparently with the rapid establishment of the necessary P. D. between stimulated and non-stimulated areas, and hence of a local bioelectric current, the condition for propagation arises.

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[5] A brief qualitative account of mechanical summation in the passive iron model is given in my recent paper (37, p. 134). Summation can also easily be shown for electrical activation, using, *e.g.*, the contact of a copper wire to produce a brief local current. For an exact study of the summation-interval in living tissues *cf.* Keith Lucas (38).

[6] *Cf.* Hill (43) for a discussion of the possible conditions in the living tissue.

It is a familiar fact that the same irritable tissue (*e.g.*, nerve) may be stimulated by many different agencies, mechanical, electrical, thermal, chemical, osmotic; *i.e.*, all originate the same self-propagating state of excitation. Why this should be the case becomes clear on the theory that in all forms of stimulation the electrical factor is the essential. Any sufficient mechanical, chemical or other alteration of the cell-surface is known to produce a local electrical negativity, as may readily be shown in muscle and nerve; a local bio-electric current (injury current, alteration current) arises between the altered region and the unaltered regions beyond. If we assume that the spread of the excitation-state, and with it the stimulation of the irritable element as a whole, depends primarily on the effect produced by the local electric current formed at the original area of stimulation, the whole problem at once assumes a more definite as well as simpler aspect. It resolves itself into the problem of the conditions of electrical stimulation in general. The current passing between the locally altered area and the unaltered area adjoining initiates "secondarily" electrical excitation in the latter region; and since any excited region becomes also locally negative, a similar current at once arises between this region and the one immediately beyond, which is then similarly excited. By a repetition of this effect at each newly formed active-resting boundary the excitation-wave travels over the entire irritable element, and the whole system is activated.

The problem of electrical stimulation can be considered only briefly in this article, which is concerned with the general conditions of transmission rather than with the special nature of the local effect produced by the current. It is now agreed that a change in the electrical polarization of the limiting semi-permeable boundary-layer of the irritable cell or element (protoplasmic surface-film or plasma-membrane) constitutes the primary change in electrical stimulation. This theory has been established on a firm foundation by the work of Nernst (41) and his successors, especially Lapicque (42), Lucas (39) and Hill (43); and the quantitative conditions of electrical excitation are considered in detail in the papers of these authors. The results of this and related work have shown that the current effects local stimulation *1*, when it has a definite *direction* relatively to the cell-surface, such as to effect a diminution of the preëxisting or physiological polarization;[7] *2*, when it has more than a certain minimal (threshold) *intensity; 3*, when it flows for more than a certain minimal *time;* and *4*, rises to its full intensity at more than a certain *rate*. The essential condition for electrical stimulation is thus a critical change of polarization (a depolarization (44)), to produce which requires a certain minimal intensity, duration and rate of change in the stimulating current.

These latter conditions differ in a significant manner from those required to produce a given change of polarization (*i.e.*, to establish a given P.D.) across a non-living partition set in the path of a current, the case considered by Nernst (41). In this case a weak or slowly increasing current, if it lasts long enough, may produce the same change of polarization as a stronger current lasting for a brief time, a condition formulated in Nernst's "square root law" ($P = Ki\sqrt{t}$).[8] The difference between the dead and the living system indicates that in the membranes of the irritable tissue processes are acting whose general direction or effect is such as to oppose or compensate the processes set in action by the stimulating current; normally the effect of these compensatory processes (presumably processes of metabolic construction, at least in part) is to bring the tissue back to the resting state after stimulation and to keep it in the normal irritable state in the intervals between stimulation. The stimulating agency must act at a greater rate than these compensatory processes, and last long enough to offset their effect, or no stimulation results.[9] A condition of this general kind is found also in the passive iron wire in strong nitric acid, where the oxidizing action of the acid keeps the passivating surface-film continuous and automatically repairs slight interruptions (*i.e.*, those insufficient to start activation-waves); the same rapidly acting oxidative process also reforms the film after the passage of an activation-wave. In this system also a rapidly increasing current of more than a certain duration is required for activation; in fact the above four rules of electrical stimulation apply also to the case of electrical activation of passive metals (33).

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[7] This is the necessary inference from the law of polar stimulation (excitation at cathode, inhibition at anode on make of constant current, and *vice versa*), as pointed out by Brünings (44).

[8] P represents polarization produced, i the intensity and t the duration of the polarizing current.

[9] Compare the discussions of Lapicque (42) and Hill (43) on the nature of the conditions necessitating a certain minimal rate of change in the stimulating current.

Apparently any rapid local decrease of surface-polarization (sufficient in range) causes stimulation in the typical irritable system such as muscle or nerve. This purely physical change, however, is merely the precursor or determinant of the local stimulation-reaction; it is not the reaction itself. The latter is a physiological process dependent, like all such processes, on chemical reactions, and having its own special peculiarities (rate, duration, chronaxie, specific features) in each irritable tissue (39), (42). The general problem is why this process should be initiated by a change of electrical polarization at the cell-surface. The only general inorganic analogy is that of electrolysis. In this case the establishment of a sufficient uncompensated potential-difference between electrode and solution forms the condition of the chemical change. When the electric current is free to pass (*i.e.*, with closed circuit) there is transfer of electricity (electrons) between electrode and solution; at the interface electrons are added to or abstracted from the molecules or ions there present, with chemical combination or decomposition as a result. We have seen that in the metal-electrolyte combination regarded above as affording a generalized model of protoplasmic transmission—passive iron in nitric acid—a local electrolysis, resulting, *e.g.*, from the contact of zinc or from a mechanical interruption of the oxide coating, forms the primary condition of a rapidly propagated wave of chemical and electromotor change also depending on electrolysis; and attention has been called to the many significant resemblances between this process and protoplasmic transmission. The implication is that in the transmitting protoplasmic system also the local bioelectric current produces, by a process essentially identical with electrolysis, chemical alterations in the surface-film, and that the general tendency of excitation-processes to spread in living protoplasm is a result of this condition, which is essentially similar to that existing in the inorganic model. At present the special chemical conditions at the protoplasmic interfaces are unknown, but in their general features the two processes give unmistakable evidence of belonging to the same physico-chemical class.

According to this conception, protoplasmic transmission is an electrochemical effect, depending on the formation of a circuit between the altered area and the adjoining unaltered area. For a certain distance (*e.g.*, R_3 R_4, fig. 1) beyond the boundary between these areas the current of the local circuit is sufficiently intense to effect the required electrolytic alteration of the surface layer; in the case of the passive wire this alteration consists in the reduction of the passivating oxide film to a lower state of oxidation; in the case of the protoplasmic system its precise nature is unknown. Any such effect is self-propagating because between the newly altered area and the area beyond a similar circuit is formed, and the same effect is repeated at each new boundary. Since the essential condition for propagation is alteration of the interfacial

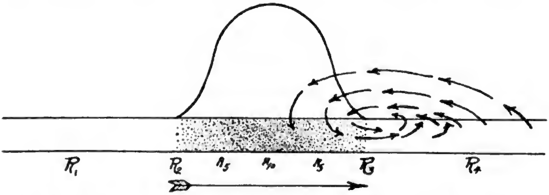

Fig. 1. Diagram of the momentary conditions in a frog's motor nerve at 20°. The shaded region marked *A*, between R_2 and R_3, is occupied at the instant under consideration by the excitation-wave, which is regarded as advancing in the direction of the large arrow at the rate of 30 m. per second. Its length is 6 cm., assuming the total duration of the local process (as indicated by the duration of the local bio-electric variation) to be 0.002 second. The excitation-process is just beginning at R_3, has reached its maximum at A_{10}, and has just subsided at R_2. The curve indicates the variation from the resting potential at different points in the active region; the maximum P. D., at A_{10}, is 30 to 40 millivolts. The unshaded regions marked *R* are in the resting state. The small arrows indicate the general direction of the bio-electric current (positive stream), in the external medium and in the protoplasm, in a portion of the circuit at the active-resting boundary. For a certain distance beyond the boundary (R_3R_4, probably about 3 cm.) the intensity of this current is sufficient to excite the nerve; excitation is thus in process of initiation in the still resting region of the nerve for this distance in advance of the wave-front. For a somewhat similar distance R_1R_2 in the wake of the excitation-wave the nerve is refractory to stimulation.

film at some distance from the already altered area, by means of the electrochemical action of the local current, it is evident that the possibility of transmission depends in the first instance upon the "chemical distance-action" effect (15, p. 165). As each new active-inactive boundary is formed the same situation arises; there is always, therefore, extending for a certain distance in advance of the already altered region a region within which the electrochemical reduction is in progress. The length of this interval, and the rate at which the film is electrochemically removed or altered, are the two chief factors determining the velocity at which the reaction is transmitted along the surface (27), (28).

This general description is based more particularly upon the conditions in the passive iron system, where the chemical conditions of transmission are comparatively simple and readily understood. In the case of a protoplasmic system like nerve, the precise nature of the chemical reactions determining transmission is unknown. There is little or no change of temperature accompanying the passage of a nerve impulse (45), but the significance of this fact is obscure;[10] possibly heat-production and heat-absorption balance each other in the local reaction. It seems probable that oxidation-reduction processes are concerned in many transmissions, and in this case the local bio-electric circuit may represent essentially an oxidation-reduction circuit.[11] The majority of stimulation and transmission processes are directly or indirectly dependent on oxygen-supply; but whether molecular oxygen enters into the local reaction in its initial destructive phase, or in its later reconstructive or synthetic phase, is uncertain. More probably oxygen is especially required in the construction phase, during which the altered surface-film is renewed (37). Nerve is not exhausted by stimulation except in the absence of oxygen (46a), (47); this condition is also known to prolong the refractory period (47), which apparently represents the period of reconstruction of the surface-film (37). In general, the above analogies with the metallic system point to the conclusion that the essential process in the local excitation-effect consists in a breakdown of the protoplasmic surface-film, under the electrochemical influence of the local bio-electric current, followed by its reconstruction under similar influence,[12]—which becomes oppositely directed as soon as the direction of the local "diphasic" current changes.[13] The process is

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[10] It cannot be explained until more is known of the nature of the local current-producing reaction. It is well known that certain electrochemical combinations abstract energy from the surroundings; according to Bernstein and Tschermak this is also true of the electric organ of the torpedo (*cf.* Bernstein, 86, chapter 6). The energy of the local bio-electric current in a nerve is insufficient to produce an appreciable change of temperature (*cf.* Hill, 48).

[11] There is some evidence (regarded as inconclusive by many physiologists) of an increased production of CO_2 by nerve during activity (*cf.* Waller 45a, Tashiro 45b). The absence of any increased rate of change in the pH of the medium bathing the nerve during activity, as reported by Moore (46) is not necessarily inconsistent with this result, since there is the possibility that basic compounds (*e.g.* NH_3) may be freed from the nerve at the same time.

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[12] The terms "breakdown" and "reconstruction" may seem to imply a more thorough-going alteration than actually occurs in the surface-film of the irritable element during the passage of the excitation-wave; but in our ignorance of the details of the process they may be regarded simply as indicating the general character of the two phases of the reversible or cyclical process at the excited area. Apparently the breakdown ("catabolic" change) corresponds to the rising phase, the reconstruction ("anabolic" change) to the return phase of the electric variation; on this view the physiological conditions have a general correspondence with those in the metallic model (compare the earlier view of Hering: Theory of the Functions in Living Matter, Lotos, ix, Prag, 1888, translated in Brain, 1897, xx, 232).

[13] Some light appears to be thrown on this problem by the effects of change of temperature. It is probably significant that the temperature-coefficient of transmission in muscle and nerve is definitely lower than that of the refractory period, which represents the time occupied by the process of restoration immediately following the passage of an excitation-wave. According to the recent results of Adrian (49), the temperature-coefficient of the rising phase of the bioelectric variation also appears to be distinctly less than that of the declining phase. Now the rising phase of the electromotor variation, in both the passive iron model and the living tissue, is determined (on the present evidence) by the breakdown (or increase of permeability) of the surface-film. Transmission is a direct consequence of this effect, as already indicated. The return or declining phase (in which the passive or resting potential returns) represents the period in which the surface-film is reformed. The results of Maxwell (50), Snyder (51), Lucas (52), Woolley (52a) and Harvey (52b) all indicate a Q_{10} of about 2 for the transmission-rate in muscle and nerve; while for the refractory period the most exact determinations (Bazett (53), Adrian (49), (54)) give values of 3 or more, a coefficient similar to that of growth processes.

On the theory that transmission is dependent on breakdown of the surface-film, and recovery on its reconstruction, it seems possible to explain this difference. Reconstruction is essentially a matter of metabolic synthesis and should have a purely chemical temperature-coefficient; while breakdown is probably mainly the result of physical disintegration, following some initial chemical alteration. Let us assume that the total period of breakdown at 20° has a duration of $3\ \sigma$, and that one-third of this period ($1\ \sigma$) is occupied by the initial chemical decomposition ($Q_{10} = 3$) and the remaining two-thirds by a physical disintegration (with $Q_{10} = 1.3$, the value for diffusion-processes). The duration of the total process at 10° would then be $1\sigma \times 3 + 2\sigma \times 1.3 = 5.6\sigma$. The ratio of the self-propagating because in a film-covered system like protoplasm it cannot occur without the production of potential-differences, and hence of local circuits which produce chemical effects involving similar alterations of film-structure in regions adjoining.

In considering cases where the detailed nature of the chemical processes is obscure, as in nerve, a more generalized conception of the above general type of situation may be desirable. Let the two parallel lines in figure 2 represent the contour of a filament, wire, or other conducting structure, covered with a chemically alterable surface-film of uniform composition and immersed in an electrolyte solution. So long as the system is undisturbed, the conditions at any two points, *e.g.*, *A* and *B*, are chemically and electrically symmetrical; the interfacial P.D. is the same at all portions of the surface, hence no current flows through the circuit if the two points are connected through a galvanometer. But if now one region (*e.g.*, *A*) is altered—chemically, mechanically, thermally, or osmotically (*i.e.*, by a concentration change)—the two regions are no longer symmetrical and a current flows; if this current produces through its electrochemical effect an alteration at *B* corresponding to that at *A*, transmission will result.

Fig. 2

It is evident that such a generalized schema may apply to systems of the most widely varying chemical composition. The details of the conditions in any system like nerve can be determined only by special

velocities at the two temperatures, 5.6/3, or 1.9, is similar to that observed for transmission velocities in nerve. Thus if transmission is due to a disintegration effect, following a brief initiatory chemical effect, a low temperature-coefficient is to be expected.

A further indication that the two processes are essentially different in character is seen in Lucas' observation (55) that a nerve may exhibit a normal rate of recovery in solutions of alcohol which greatly retard transmission. Transmission and recovery are thus differently affected by both temperature and chemical reagents. It is known that the refractory period may be artificially lengthened by conditions (poisons and lack of oxygen) which have little effect on transmission or on the rising phase of the bio-electric variation (93), (94), (95). In the passive iron model the duration of the recovery phase may be varied within wide limits without affecting the rate of transmission, by simply varying the concentration of the HNO_3 (37).

investigation; but it is to be remembered that the possibility of transmission of the type under consideration is determined by the *general* features of the system and not by its special peculiarities of composition and structure. The essential condition in all cases is the presence of a thin, chemically alterable surface-film separating two media, both of which conduct electricity, the passage of electricity between the two media being attended with chemical and structural alteration in the film.

There is in fact much evidence that stimulation processes are accompanied by alteration or temporary breakdown (associated with permeability-increase)[14] of protoplasmic film-structure, especially of the limiting surface-films or plasma membranes (56), (57), (27). The fundamental theoretical importance of such evidence will be clear from the foregoing, and a brief review of the chief known facts will indicate how widespread such processes are in living protoplasm. In its elementary physico-chemical constitution protoplasm is most closely related to the emulsions or emulsion-like systems, as Bütschli recognized many years ago (58); and, as in other such systems (35), interfacial films are undoubtedly a chief factor in determining its normal physical properties and especially its structural stability, *i.e.*, in preserving the normal subdivision and distribution of the component phases (59), (60). In many instances, the protoplasmic emulsion is highly unstable. The platelets of mammalia and the "explosive corpuscles" of crustacea (61) are well-known instances; here slight chemical or mechanical changes lead to a rapid and complete disintegration of protoplasmic structure. Many striking instances of this type of effect have been brought to light since the introduction of the methods of micro-dissection. Leucocytes, red blood-corpuscles, germ-cells and other cells not ordinarily classed as "irritable," when cut or punctured locally by a capillary needle, often exhibit a spread of disintegrative alteration which may involve the whole protoplasm (62). The protoplasmic boundary-film loses its normal semi-permeable properties, and this effect spreads; a good illustration is Chambers' observation that a red corpuscle pricked at one point immediately shows loss of hemoglobin over its whole surface (63). Recently many interesting instances of progressive disintegration of protoplasmic structures, including membranes, following local injury have been observed in Protozoa by Taylor (64). In other cases the injury may remain localized, its spread being delimited by the formation of a new surface-film; this phenomenon is frequent in echinoderm egg-cells (65). The spread of such disintegrative effects varies according to the conditions, *e.g.*, with the extent of the local injury or the state of the protoplasm. In the isolated nerve-ganglion cells of the lobster Chambers found that more than a certain degree of mechanical injury resulted in a complete and irreversible change in the properties of the protoplasm. "When this limit is passed the viscid plasma sets into a coagulated non-viscous mass which may be broken into non-glutinous pieces" (66). Loss of mechanical coherence, usually associated with coagulative changes in the cell-proteins, is a frequent effect of irreversible protoplasmic alteration of this kind; this is seen, *e.g.*, in the death-rigor of muscles. A similar change occurs as a result of excessive stimulation in certain irritable and contractile forms of protoplasm, *e.g.*, the large compound cilium or swimming plate of ctenophores (67). When these structures are placed in certain solutions (*e.g.*, pure isotonic NaCl) there follows a marked and striking acceleration of contractile or vibratile activity during which the normally clear and translucent protoplasm undergoes a progressive whitening or coagulation; both processes cease within a minute or two, by which time the protoplasm has lost its structural coherence and has become permanently opaque and coagulated. Evidently the physical state (subdivision, etc.) of the structural proteins is irreversibly changed as a result of some process associated with intense stimulation. It is especially noteworthy that during the excessive activity resulting from this treatment the individual fibrils or cilia composing the plate frequently lose coherence and vibrate independently. Apparently the normal interstitial material, corresponding to an interfibrillar film-structure, is irreversibly broken down during the continued excessive activity resulting from the abnormal stimulation. It is to be presumed that during the normal rhythm of contraction this film-structure is alternately broken down and replaced in such a manner that the restoration during each contractile cycle is complete, but that under the above abnormal conditions the process of reconstruction is imperfect and progressive disintegration results.

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[14] A breakdown would imply interruption of continuity and hence increase of permeability, of which there is much evidence (56).

The ability of cut or injured protoplasmic surfaces to form new film-structure has long been known; this property is highly significant in relation to the power of repair and maintenance possessed by all forms of protoplasm. In the recovery of the irritable cell or element after stimulation this film-forming process is almost certainly concerned, as indicated especially by the phenomena of the refractory period above referred to; in certain egg-cells (Echinarachnius) a process of apparently the same kind can be shown to occur immediately after insemination (36). In those cases where the breakdown of the protoplasmic structure is more rapid than can be compensated by the succeeding reconstruction the effects of stimulation may be irreversible; this appears to be exemplified in the above cited instances of the lobster ganglion-cell and the ctenophore swimming-plate; structural breakdown from exhaustion is in fact well known in higher as well as in lower organisms.[15] A recent review by Seifriz (69) gives many instances of this capacity of living protoplasm to form new films or plasma membranes at cut or altered surfaces.

In any irritable and conducting cell or element altered by normal stimulation, the formation of a new surface-film[16] apparently occurs at each region immediately after the passage of the activation-wave. If this conception is true, the general conditions in living protoplasm resemble those existing in the passive iron model in strong HNO_3, where as the activation-wave passes each region there is a chemical and physical disintegration of the surface-film followed by its reformation. This surface change is accompanied by a variation of potential, and hence by the formation of a local current which determines the transmission in the manner already indicated. The evidence that stimulation is associated with a reversible increase in the permeability of the limiting protoplasmic membranes has been reviewed in several of my former papers on stimulation (27), (56), (70). A striking instance where the transmission of the activating effect is clearly accompanied by a rapidly reversed breakdown or loss of continuity in the protoplasmic surface-layer has recently been observed by Just (36) in the sand-dollar egg (Echinarachnius) during insemination.[17] At the point of sperm-entry a characteristic local process, associated with the separation of the fertilization-membrane, is initiated in the surface-layer or "cortical zone" of the egg. This change travels slowly over the cell-surface (diameter = *ca.* 140μ) and reaches the opposite pole some 20 seconds later. As it travels the protoplasmic surface-film loses temporarily its normal coherence or tenacity, so that if the egg is then placed in dilute sea-water (*e.g.*, 60 *vols.* fresh *plus* 40 sea) it rapidly swells and bursts, the disruption beginning at the region to which the alteration-wave has extended. This unstable and non-coherent state of the plasma-membrane lasts at each region for only a few seconds, and within about one minute after insemination the whole egg has recovered its normal resistance; it then simply swells without bursting in sea-water of this dilution. These appearances indicate that as the change passes over the egg-surface the external layer of protoplasm undergoes a local disintegrative alteration, losing coherence and semi-permeability; at the altered region a thin film of surface-material is lifted off as the fertilization-membrane; following this process a new coherent and semi-permeable surface-layer is rapidly reformed. In its general features this process resembles that accompanying cytoplasmic cleavage in this egg and in the Arbacia egg, where during the formation of the cleavage-furrow the plasma membrane changes its properties in such a manner that the eggs break down rapidly in sea-water of the above dilution; this medium has no such effect in the intervals between cleavage (71). Such a reversible surface-change is probably closely similar to that accompanying the transmission of an activation-wave along an irritable element like a nerve-fiber, with the difference that the processes of local breakdown and reconstruction are much more gradual.

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[15] Many striking instances are described in the recent book of Crile (68) on shock and exhaustion.

[16] Of course the degree to which this regeneration involves complete replacement, or simple repair of an altered though permanent structure, cannot be said at present. In the passive iron model an entirely new layer is apparently formed.

[17] Certain details, soon to be published, are included in the following description, with the kind permission of Doctor Just.

In most of the foregoing examples of protoplasmic transmission the reaction determining the propagation is apparently confined to the surface-film bounding the cell or cell-element from the external medium. Similar transmissions, however, undoubtedly occur within the interior of single cells; and recently an experimental study of these intracellular conduction processes has been made with the microdissection apparatus by Taylor (64), using the ciliated protozoön, Euplotes. In this form there are present certain definite intracellular non-contractile protoplasmic strands or fibrils[18] which are connected both with the external motor organs (cirri and membranelles) and with an internal central structure, the "motorium," to which an integrative or coördinating rôle is ascribed. Cutting these various tracts was found to produce definite disturbances of coördination and conduction. It thus appears probable that within the limits of single cells systems of intracellular tracts or filaments may exist, transmitting influence from region to region. The intracellular fibrils of nerve-cells belong probably in this category. If such filaments are bounded by films similar in properties to those bounding the general cell-surface and continuous with the latter, excitatory or similar influences originating at the cell-surface may be thus transmitted through the cell-interior and there modify chemical or other processes. Such a conception would regard the internal protoplasm of many irritable cells (muscle-cells, gland-cells, photogenic cells, etc.) as pervaded by a system of film-structure similar in properties to that enclosing the entire cell; both the structural and physiological characters of the protoplasmic system would then vary with the physical and chemical state of these films.[19] Thus any irreversible breakdown of this film-system would lead to structural and chemical disintegration, as in the cases cited above; but a temporary and reversible alteration, similar to that assumed to occur in the surface-film of a conducting neurofibril, would have a temporary and reversible effect which would cease when the film-structure was reformed.

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[18] These strands are similar to those first described by Engelmann (72) in Stylonychia in 1880, and to which he attributed a conductive function.

It appears probable that in contractile fibrillar forms of protoplasm, *e.g.*, muscle-cells, cilia, membranelles, swimming plates, processes of this type form an essential condition of contraction. Removal or alteration of the interfacial film between fibril and sarcoplasm would alter the surface-tension and produce mechanical effects in a manner similar to that seen during the removal of the interfacial film in the $Hg - H_2O_2$ system (29). It is clear that in a stimulated muscle cell the effect of an alteration originating at the cell-surface is propagated through the entire protoplasm and causes contraction in all of the fibrillæ. That the contractile process in the ctenophore swimming plate is associated with a temporary breakdown of the interfibrillar material is indicated by the effects described above as occurring in pure NaCl solutions, especially the loss of coherence between the fused cilia forming the plate; many of these vibrate independently during the brief period of excessive activity, at the same time undergoing a coagulative alteration. The whole appearance is strongly suggestive of a rapid and irreplaceable breakdown of some interstitial material or film-structure which is essential to the normal properties of the whole plate and forms the medium of the coördinated movement of its separate fibrils. This conception corresponds essentially to that of a continuous film-system representing the conductile part of the protoplasm, normal conduction depending on its local breakdown followed by its prompt reconstruction.

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[19] Compare Hofmeister's conception (73) of a chambered or film-partitioned structure (related to emulsion structure) as controlling chemical reactions in cells. For example, the rapid onset of autolysis at death may be due to the removal of a film-structure which normally limits the interaction of the intracellular enzymes and their substrates.

The temporary interruption of such intracellular films would permit chemical interaction between compounds which in the normal resting state of the protoplasm are kept apart; and in fact many instances are known where reactions which occur slowly or imperceptibly in the intact cell proceed rapidly when protoplasmic structure is destroyed, or after natural death; the oxidase reaction which causes browning in fruits, potatoes and leaves, and the reactions of autolysis are examples. The recent investigations of Harvey (74) on light-production in animals are especially interesting because of the indirect light which they throw on the inner mechanism of stimulation processes. The substances luciferin and luciferase, whose union in the presence of oxygen determines light-production, are apparently both present in the irritable photogenic cell, but in many organisms their interaction occurs only as the result of stimulation. Evidently some physical barrier preventing this union is then temporarily removed. The sensitivity of a luminous unicellular organism like Noctiluca to mechanical or electrical stimulation is most readily understood on the assumption that an intracellular film-structure, which is broken down and replaced in stimulation, controls the photogenic reaction. Light-production thus forms an index of stimulation in the same sense as the bio-electric variation, both processes depending on alterations in the protoplasmic film-structure.[20] The phenomena of irritable gland cells, which show both increased permeability and bio-electric variations during stimulation, are further examples having a similar significance.

The evidence is conclusive that in the surface-films covering passive iron in HNO_3 or mercury in H_2O_2 the progress of the wave of disintegration is the direct result of electrolysis by local circuits. The evidence that transmission in protoplasmic systems is also a consequence of the local electrical currents produced in excitation is mainly of an indirect nature. The above parallels between the processes of activation and transmission in the metallic models and in living protoplasm are, however, of a kind otherwise difficult to explain. The presence of thin alterable interfacial films seems to be the only significant structural feature possessed in common by both systems. Such considerations lend a new significance to the familiar fact that the physiological effects produced by external electric currents, *e.g.*, in muscle, are indistinguishable from those produced by normal innervation. On the present interpretation normal innervation represents in reality a form of electrical excitation. It has long been known that the passage of an activation-wave in nerve or muscle is associated with a local electrical variation and circuit (76); and in any electrically sensitive film-partitioned system of the type of living protoplasm such currents must have chemical efforts wherever they traverse the phase-boundaries.

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[20] An interesting instance of transmission of a light-producing reaction in the colonial cœlenterate animal Renilla has recently been described by Parker (75). Waves of light travel over the surface of the organism from the point of stimulation. The Q_{10} of this transmission is *ca.* 2.

It is clear from inspection (see fig. 3) that the local bio-electric current accompanying the activation-wave, *e.g.*, in a nerve, passes in such a direction that its normal effect—if it causes stimulation under the same conditions as other electric currents—would be to excite the resting areas adjoining the active area, and repress excitation in the active area. Also we know that the action-currents of a tissue have the intensity,duration and rate of change required to excite that tissue ("rheoscopic frog" experiments, etc.). These phenomena of secondary electric stimulation are apparently shown in isolated cells like spermatozoa (77) and ciliated cells, which beat synchronously when in close contact with one another. The synchronous vibration of flagella in protozoa is undoubtedly a closely related phenomenon. The view that transmission of excitation is essentially a case of secondary stimulation by

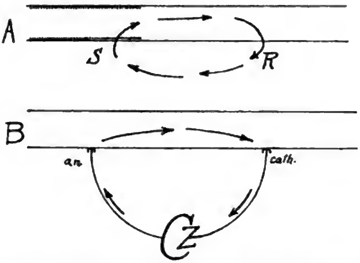

Fig. 3. In *A* the arrows represent the direction of the bio-electric current in the local active-inactive (or action current) circuit on one side of the stimulated or active region *S* (dotted lines). In *B* the course of an external stimulating current from a battery is represented. Stimulation originates, on make, at the cathodal electrode, where the current has the same direction, relatively to the protoplasmic surface, as at *R*.

bio-electric currents was in fact long ago entertained by du Bois-Reymond (78), Hermann (79), Kühne (80), and other earlier students of the bio-electric phenomena. Du Bois-Reymond even speaks of the possibility that processes of electrolysis may underlie electrical stimulation (81).

Many other facts indicate the essentially electrical nature of the factors determining transmission. Thus it is well known that both local stimulation and transmission may be facilitated or hindered by the passage of constant currents through the tissue; this effect is definitely polar (electrotonus), and probably represents in large part an interference effect between opposed electrical currents (37, pp. 136-138). The effects of local injury in blocking nerve transmission may belong in the same category, the local injury current compensating the current of the normal bio-electric circuit as the activation-wave nears the region of injury. Further evidence that the local action-current forms the essential condition for transmission is seen in the effects of altering the electrical conductivity of the medium. Mayor found that in dilute sea-water the rate of transmission in the nerve-net of the medusa Cassiopea is less than the rate in normal sea-water, the decrease for a given dilution running closely parallel with the decrease in the electrical conductivity (82). The relation between the conductivity of the medium and the rate of transmission of the contraction-wave in muscle has recently been made the subject of a special investigation by Pond (83), using the Limulus heart and vertebrate skeletal and cardiac muscle. The normal medium (sea-water, Ringer's solution) was diluted with isotonic sugar solution, and within a wide range of dilutions a close correlation between the electrical conductivity of the altered medium and the rate of transmission was found. This correlation is to be expected if electric currents traversing the extra-protoplasmic medium are in fact an essential factor in transmission.[21] The distance (from the already active area) at which the bio-electric current traversing the resting area has an intensity sufficient for stimulation must decrease (other conditions being equal) as the conductivity of the local circuit is decreased; this implies a corresponding decrease in propagation-velocity. One reason why the presence of salts in the tissue media of higher animals is essential is probably that they furnish the electrical conductivity without which rapid transmission would be impossible. The further fact, already cited, that the speed of propagation exhibits a close correlation with the rate at which the local variation of potential rises to its maximum, also indicates that propagation is determined by the current of the local active-inactive circuit. The more rapidly this current comes into existence, the more rapid must be its secondary stimulating effect in adjoining areas and the more rapid the consequent transmission (28).

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[21] The fact that stretching a nerve (within moderate limits) leaves its rate of transmission unaltered (96), (97) probably has a similar significance. Excitation is always being initiated in the resting region of the nerve by the current of the local active-resting circuit up to a certain distance in advance of the already active area; on the present theory this is the distance at which the strength of the current where it traverses the surface-film just reaches threshold value, and is determined by the electric resistance, *i.e.*, the *length*, of a column of solution extending beyond the active area for the distance in question.

One further fact of much theoretical interest is that the phenomenon of transmission with decrement, briefly described above for nerve, is exhibited in essentially the same form in the passive iron model as in the living tissue (37). This type of transmission is shown immediately after the wire has undergone complete activation in strong HNO_3 (*e.g.* > 1.25) and has reverted to the passive state; a certain time is then required for the recovery of complete transmissivity; at first an activation-wave initiated at any point travels slowly and for only a limited distance along the wire; by degrees both the rate and distance of transmission increase, and eventually—*e.g.*, in 75 per cent HNO_3 (*sp. gr.* 1.42) after about 10 minutes (at 20°)—the wire transmits for an indefinite distance as before. Evidently the condition of the newly formed passivating surface-film is such that it is less readily and completely altered or removed by the current of the local circuit than a film which has been exposed to the acid for some time. If the analogy with protoplasmic transmission applies also to the conditions determining transmission with decrement, it is to be inferred that the action of any agent, *e.g.*, an anesthetic, which converts unlimited transmission in a living system (such as nerve) into transmission with decrement, depends upon its altering the properties of the protoplasmic surface-film in some definite manner. On the theory that the normal properties of the irritable living system, especially the normal susceptibility to electrical stimulation and the dependent property of transmitting excitation-waves, are determined by the properties of the surface-films, we should expect that when by any means these structures are rendered less alterable than before, *i.e.*, stabilized, by the action of some physical or chemical agent, activation-waves will fail to be transmitted freely and the system as a whole will then exhibit itself as non-irritable. Reversible effects of this kind apparently form the basis of anesthesia, narcosis, and similar reversible inhibitions or suspensions of irritability.[22]

Elsewhere I have attempted a more detailed comparison between the phenomena of activation, transmission and recovery in the living system and in the passive iron model (37). To the electrochemist the possibility of such a comparison may seem doubtful, because in all artificial systems hitherto constructed in which electric currents are produced by chemical action—and in which conversely chemical action is produced by the passage of a current—an essential condition appears to be the combination of both metallic and electrolytic conductors, so arranged as to form a complete circuit. Chemical action is confined to the regions where electricity passes between the one type of conductor and the other—*i.e.*, to the layer of molecules at the boundary-surface. Living protoplasm presents conditions similar to these in some respects and widely different in others. No metallic phase is present; the essential arrangement appears to be a combination of electrolytic conductors partitioned by thin semi-permeable films consisting essentially of chemically alterable colloidal material. Yet chemical reactions are produced in this system by the passage of electric currents; and in its normal activity the system gives rise to electric currents. The bio-electric potentials are small (*ca.* 0.05 to 0.1 volt) in the case of single cells, but they may be summed when the elements are superposed, as exemplified especially in the electric fishes.[23]

If in reality the conditions determining the electrical behavior of the living and the non-living systems are fundamentally of the same kind, it would appear necessary to assume that the semi-permeable films of living protoplasm behave in some way as conductors of the first class. There seems to be no theoretic impossibility in this, and investigation of the electrical conductivity of the water-insoluble compounds present in these films (*e.g.*, lipoids, certain proteins, Ca-soaps) might yield important results, although it is improbable that such substances have more than the low conductivity usual with organic compounds of this type (oils, rubber, etc.). What is of chief physiological interest is the evidence that the primary effects of electrical stimulation are at the protoplasmic boundary-surfaces; apparently it is because of the chemical effects there produced by the current that the surface-films undergo alteration (of permeability, electrical polarization, physical consistency, etc.) during stimulation and transmit waves of chemical change to a distance. The above difficulty largely disappears if we assume that the distinction between conduction in a mass of metal and conduction across a partition of "non-conducting" material loses its importance when the material is in a sufficiently thin layer. Such an assumption is not merely arbitrary, since it is probable that all substances, even water, have some degree of "metallic" conductivity; hence conduction through a layer of oil only a few molecules in thickness need meet with no more resistance than conduction through a thick layer of carbon, metal, or other conductors of the first class. The conditions at the contact of such a thin film of "non-conductor" with an electrolyte solution during the passage of a current between the two would then be essentially the same as at the surface of a metallic electrode. What is essential is that a current should pass across the boundary between the two conductors.

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[22] The evidence that these conditions depend primarily upon alteration of the protoplasmic surface-films or plasma-membranes is summarized in my review, *The Theory of Anæsthesia* (84).

[23] For summation of bio-electric potentials *cf.* Brünings (85). The essential structural peculiarity of the electric organ is the arrangement of the elements (plates, discs) in series, with the innervated and non-innervated surfaces of successive elements adjoining each other. This seems to be a clear indication of the electrode-like properties of the surface-layers. *Cf.* Bernstein (86) for a fuller account and literature; also Gotch (87), Biedermann (88), Garten (89a).

In electrolysis the chemical reaction is confined to the layer of molecules at the *boundary*, where electrons are transferred between the phases. As regards the electrochemical processes occurring at the boundary region it is a matter of indifference whether conduction in the *interior* of either phase is metallic or electrolytic; this can readily be seen if we imagine the metallic phase reduced to the thickness of a few molecules; only the surface molecules are concerned in the chemical process in any case. Adsorption films of oil or similar material need be only one or two molecules thick (89); and in living matter adsorption is undoubtedly one of the chief preliminaries to chemical reaction (90). To explain the behavior of living matter with reference to the electric current, therefore, it seems only necessary to assume that the chemically reactive material is present as an adsorbed layer of molecular thickness at the surface of a thin continuous, water-insoluble film, having a conducting aqueous phase (salt solution) on either side. The passage of a current across such a film would be attended with chemical alteration, and, conversely, chemical alteration (*e.g.*, oxidation of the film material) would yield a current. The plasma membrane, together with the internal protoplasm and external medium adjoining, apparently represents an arrangement of this type.

On the other hand, if the living system, in its current-producing and electrosensitive capacity, represents a special type of electric cell as yet unknown to electrochemistry, we ought to be able to synthesize such cells artificially, instead of depending on the processes of organic growth to synthesize them for us. It seems difficult to believe, in view of the detailed nature of the above parallels, that the living and the non-living systems are fundamentally dissimilar in the conditions determining the relations between electric circuits and chemical change. There is in fact much evidence that under certain conditions the passage of electric currents across inorganic semi-permeable membranes (precipitation-membranes) is attended with chemical change (91).[24] The related facts of electrostenolysis (92) also show that metallic conductors are not necessary for the production of electrochemical effects. The more complete determination of the conditions underlying the electrical sensitivity and current-producing capacity of living matter must, however, await further investigation.[25]

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[24] An apparently similar effect is seen at the nuclear membrane of blood corpuscles in the indophenol oxidation reaction; the granules of oxidation-product are deposited most rapidly at the nuclear surface, and their formation is accelerated by induction-shocks (98).

[25] A complete discussion of this problem is not possible here. Its essentials may be defined briefly as follows. The surface of the living cell and the surface of a metallic electrode have certain conditions in common, such that the passage of an electric current between either and the adjoining electrolyte solution is attended with chemical change in the substances at the interface. In the metal-electrolyte combination the carriers of electricity (electrons and ions respectively) are different in the two phases, and each phase is impermeable to the carriers of the other phase. But with a sufficiently steep fall of potential across the boundary and a complete circuit (not compensated), electrons are transferred between the metal and the ions or molecules in contact; in other words, a current flows between solution and metal, and the molecules or ions which lose or gain electrons undergo corresponding chemical change. The chemical rearrangement may involve either decomposition or synthesis, oxidation or reduction, of particular compounds, according to circumstances. In the living cell also the surface is impermeable to the carriers, *i.e.*, a semipermeable film is interposed between medium and internal protoplasm; this condition allows a P.D. to exist across the cell-surface. The film between the two electrolyte solutions (intra- and extra-cellular) is thin and the fall of potential across the cell-surface correspondingly steep; hence when a current passes the conditions are not essentially different from those at the metallic surface; *i.e.*, there is transfer of electrons between molecules and ions at the interface, with associated chemical change. If this reasoning is correct, we may conclude that the transition from a metallic to an electrolytic conductor furnishes the same conditions (as regards the effects produced by passing a current) as the transition from one solution to another across a thin semipermeable membrane.

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ON THE ORIGIN OF THE CELLS OF THE BLOOD

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The history of the development of our knowledge of the blood centers around two opposing theories, and nowhere in medicine can there be made a more interesting analysis of the value of constructive theories, whether right or wrong, in the advance of science. No fact is better known than that the foundation of our knowledge of blood was made by Ehrlich. His introduction of the method of a chemical analysis of cells by means of dyes made modern hematology possible. Ehrlich divided the white cells of the blood into the granular or leucocytic forms and the non-granular or lymphocytic types.

In the leucocyte Ehrlich found five specific types of granulations, only three of which occur in human blood: first, the α or oxyphilic, eosinophilic granule, which is large, refractive, uniform in size, round as in human blood, or in long rods as in birds; second, the β or amphophilic granule having the power to bind a chromophilic radicle from either a basophilic or an acidophilic dye. This type of granule is found in young cells, never in the adult forms. Third, the γ granulation which is the metachromatic, basophilic granule of the mast cell; fourth, the δ granule which is truly basophilic and is not found in human blood, and fifth, the ϵ or neutrophilic granulation. This is the fine granulation which in human blood is neutrophilic, showing best in the Ehrlich triacid mixture, and in May-Giemsa stain. In some animals the fine granulation is faintly eosinophilic instead of neutrophilic and is termed pseudo-eosinophilic.

Ehrlich saw in the affinity of these different granules for specific types of dyes an expression of a true chemical reaction and an indication of the division of the cells into specific functional groups.

Since Ehrlich's work it has been shown that eosinophilic granules have a lipoidal envelope which stains in certain basophilic, vital dyes. In 1902 Michaelis and Wolff (41) discovered that there is another type of granule, stainable in azur which is to be found in lymphocytes. In both of the great modern atlases of blood, namely Pappenheim (55) (56) and Ferrata (20), only certain lymphocytes are shown with granules, and yet I think that granules can be made out in all living lymphocytes though often not more than two or three to a cell. This granule is metachromatic and Michaelis and Wolff thought that this granule was characteristic of the large mononuclear forms and the transitionals as well, but Naegeli (51), (52) showed that the large mononuclear cells and the transitionals belonged together in one group, the monocytes, in having a granule which is not identical with the azur granule of the lymphocyte. At first (1909) he thought that the granule of the monocytes was neutrophilic allying the cells with the myeloid forms, but later (1912) he found that their granule is neither azurophilic nor neutrophilic. It differs from the azur granule chemically because it gives the oxydase reaction while the granule of the lymphocyte does not. The oxydase reaction was introduced by Winkler (69), and consists in producing idophenol blue in cells that have a specific ferment to synthesize the dye from alphanaphthol and dimethylparaphenylendiamin. This ferment is in the granules of the leucocytes and monocytes but not in the lymphocytes.

Ehrlich's classification then led to six types of white cells, the neutrophilic, eosinophilic and basophilic leucocytes, the small lymphocytes with which were included certain intermediate forms, the large lymphocytes or mononuclear forms and the transitionals. The studies on the blood since the time of Ehrlich have led up to a classification of the white cells into three groups; first the leucocytes with their three different cells; second the monocytes, with two different types, large mononuclear forms and the so-called transitional cell and third, lymphocytes. By this classification I mean to indicate specifically the absence of any transitions between the cells of the three main groups, leucocytes, monocytes and lymphocytes.

In the studies of blood and bone marrow made by Ehrlich and following his work, three problems dominated. First, the question of how primitive nucleated red cells became the non-nucleated red blood corpuscles, through tracing the young erythroblasts with large nuclei having comparatively little chromatin, through the stages of the normoblast with a smaller nucleus which becomes gradually pycnotic to the stage of the extrusion of the nucleus. Secondly, the study of how the various types of myelocytes in the bone marrow became the three types of leucocytes by the gradual change of their oval nuclei into the characteristic polymorphic types. Thirdly, the question of the origin of blood platelets. In connection with platelets it is now generally recognized that Wright has demonstrated that they are fragmented parts of the cytoplasm of the megalocaryocytes of the bone marrow.

The work of Ehrlich on anemias and leukemias led him to separate the two great groups of blood-forming organs of the adult, namely, the bone marrow and the lymph glands; the bone marrow giving rise to erythrocytes and the myelocytes as forerunners of the leucocytes while the lymph glands give rise to lymphocytic forms. In this system the spleen gives lymphocytes normally but readily returns to its embryonic myeloid function as a compensatory reaction in disease. This is the so-called dualistic, or more strictly polyphyletic theory of the origin of blood associated with the name of Ehrlich, and later with Naegeli, Schridde, Morawitz and others. This theory postulates that there are different stem cells for the reds, for the leucocytes and the lymphocytes and that they are located in specific places in the adult.

It is, I think, exceedingly interesting that though it may well prove that the Ehrlich doctrine is nearer to the truth than the more modern, monophyletic theory, it cannot, I believe, be questioned that most of the progress in the subject of the blood since Ehrlich has been made under the monophyletic theory. A list of the chief names associated with the theory will prove this: Dominici, Pappenheim, Weidenreich, Maximow, Danchakoff, Ferrata.

The foundation of the monophyletic school was laid in the discovery that there can be found for each type of cell in the blood—the erythrocyte, as well as for every type of white cell—a primitive cell with basophilic, azurophilic cytoplasm. Thus we pass into a period in which the study of cytoplasm was stressed more than the nuclei. The identification of this primitive cell as a common stem cell or hypothetical hematoblast is then the standpoint of the monophyletic theory. The first clear account of such a primitive, basophilic cell as a general stem cell is to be found in a paper of Pappenheim (53), in which he calls attention to the fact that Ehrlich had noted that young myelocytes had a basophilic cytoplasm while the only stainable substance of the cytoplasm of their descendants, the leucocytes, was the specific granulation. Pappenheim discovered a primitive stem cell with basophilic cytoplasm as a forerunner of erythroblasts and identified this primitive cell as a large lymphocyte. The working out of this theory has depended on the development of a new series of stains for blood, all modifications of the Romanowski eosin-methylene blue stain, namely, the entire group of azur dyes, the Giemsa stain and all its modifications, May-Giemsa, May-Grünwalt, Wright, Jenner, Wilson, etc. Azur is an oxidation product of methylene blue. Azur II, which is the basis of these stains is a mixture of methylene blue and azur. The clearest account of these methods is to be found in Naegeli's book, *Blutkrankheiten und Blutdiagnostic.*

The best account of the embryology of the blood, from the standpoint of the monophyletic school, is to be found in the work of Danchakoff (6) for birds and Maximow (35), (36) for mammals. Both use the eosin-azur stain after fixation in zenker-formol, and section the embryos in celloidin. Madame Danchakoff finds that the original blood cell is a "primitive stem cell" derived from the blood islands which is neither to be identified as a red nor as a white cell. This primitive cell develops first within the vessels and subsequently extra-vascularly and in part from endothelium. At the stage of eighteen somites in the chick part of these primitive cells have become erythroblasts so that she finds in the vessels two types of cells, namely, the primitive lymphocytes and the erythroblasts. On the end of the fourth day of incubation the granulocytes differentiate from an extra-vascular stem cell, so that the stem cell within the vessels gives erythroblasts, while the stem cell without the vessels gives the granulocytes. The essential conclusion of Madame Danchakoff's work rests on these facts, that she has discovered that the red cells of the chick arise within the vessels, while the granulocytes arise outside the vessels. The cells from which these two groups of blood cells come, look alike and, being regarded as identical, she concludes that a common stem cell gives one or the other group according to its environment. Her four plates give beautiful figures of all of the stages in the differentiation of erythrocytes, from the first appearance of hemoglobin through the stages of polychromasia, which is the name to express that in the eosin-azur dyes basophilic cytoplasm stains blue and hemoglobin stains red and both show at the same time in the same cell.

Maximow (35), (36) has given the best and most complete account of developing blood, using mammalian forms. He finds that the first blood cells of mammals develop within the vessels of the embryonic membranes as do those of the chick; that the primary cells are first lymphocytes, then erythroblasts. He notes the very close relationship of endothelium to the blood cells but thinks that the endothelium gives rise only to indifferent stem cells. Subsequently the formation of blood goes on within the embryo and in the mammal he thinks becomes entirely extra-vascular. Thus he thinks that there are two groups of erythrocytes, the primitive intra-vascular forms that disappear, and the definitive extra-vascular group that is permanent.

He finds that the formation of blood within the embryo is at first diffuse, the cells differentiating from the mesenchyme in many places, notably in the region of the head; subsequently becoming localized in the liver, in the spleen, and finally in the bone marrow. Maximow believes in a large lymphocyte as a common stem for all the blood cells. He finds that the marked difference between the chick and the mammal lies in the extra-vascular origin of the red cells in the latter, in all but the earliest stages. The extra-vascular origin of the red cells in mammalian bone marrow is now regarded as proved and is generally accepted. It rests on this work of Maximow and on the studies of Mollier (43) on the development of blood in the liver. In connection with the work of Mollier it can be shown from his own figures that the extra-vascular spaces he describes may just as well be interpreted as a part of the lumen of the developing vessels in the light of our present knowledge of the differentiation of blood vessels from a vasoformative cell, the angioblast. In connection with the origin of red cells in bone marrow, we have been up until now without complete knowledge of the anatomy of the vascular system in that organ. It has been shown in some work by C. A. Doan, in this laboratory, soon to be published, that there is a very extensive plexus of collapsed capillaries in the adult bone marrow of the pigeon. Thus the subject of the origin of red cells in bone marrow must now be re-studied in the light of this better understanding of the structure of the organ.

In 1907, Maximow (38) followed the evolution of bone marrow by stimulating the formation of bone and marrow in the kidney by tying off the renal vessels in the adult rabbit. By this experiment he obtained a marrow much more simple than that of normal development and one in which it was easy to follow the stages, which are a preliminary dilatation of the small veins giving a slowing of the circulation and the subsequent differentiation of the various types of cells from a basophilic stem cell. The earliest identifiable red cell he calls a megaloblast, which has a very large pale nucleus, then an erythroblast and finally an erythrocyte. He gives a careful description of the evolution of each type of cell and an especially clear account of the differentiation of the granulocytes by a gradual increase in the specific granulation in a primitive, basophilic stem cell.

The presentation of the monophyletic theory has been the life work of Pappenheim in Germany and of Ferrata in Italy. In 1905 to 1912 Pappenheim published his well-known atlas, and since Pappenheim's death the Folia Haematologia (56) has published his final monograph which covers the entire field of hematolgoy.

The presentation of the various forms of the blood cells in the adult and in development as they can be seen in the azur-eosin technique, in normal and pathological states in these two atlases and the two papers of Maximow and Danchakoff, cannot be surpassed. The monograph of Pappenheim covers completely all the known facts of blood and is a real mine of accurate information but made excessively abstruse by countless genealogies of blood cells which I think are all based upon a misconception of the origin of blood. With the elimination of Pappenheim's hypothetical "lymphoidocyte" all of this complexity would disappear and this complexity could indeed drop out without detracting from his achievement. The work of Pappenheim in founding the monophyletic school and in tracing the maturation of individual blood cells has led and dominated hematology since the work of Ehrlich.

The atlas of Ferrata (20) also covers the whole field of modern hematology. He brings out further stages of the evolution of the specific types of blood cells as a completion of the main contribution of the monophyletic school. His first ten plates cover the maturation of the red blood cells most completely and beautifully. He has worked out the changes in the azurophilic cytoplasm leading up to basophilic punctation in experimental lead poisoning. The most important section of his book is the one in which he deals with the relation of the blood cells to the connective tissues in general, a subject of great interest, as will appear later.

To sum up the work of the monophyletic school, they have shown that the original cell of each series of the blood cells has a basophilic cytoplasm staining especially in azur; they have given us a complete and accurate history of each specific cell; they have shown that the erythrocyte begins as a cell with basophilic cytoplasm in which the acidophilic hemoglobin gradually appears so that the young cells have a stage when both substances stain, called polychromasia, which is gradually succeeded by the ripe forms in which the only stainable substance is the acidophilic hemoglobin. They have described more fully the stages in which granulocytes begin by a development of specific granulation in a primitive basophilic cytoplasm and finally in their beautiful atlases of lithographic plates have illustrated the entire range of variation of the three types of white cells, namely the lymphocytes, the granulocytes (leucocytes) and the monocytes. The lymphocytes include all the small lymphocytes and the older cells up to those about twice the size of the small cell. The monocytes include the very large, pale, mononuclear forms, and all of the transitional forms of Ehrlich. The actual separation of monocytes (histiocytes) as a specific functional group is due to Aschoff and Kiyono. The monophyletic school have, I believe, made a mistake in the identification of the lymphocyte or a lymphocyte-like form, as a common stem cell. It is, I think, unquestionably true that the primitive stem cells of the different groups of blood-cells cannot be separated in fixed specimens even with the most perfect technique, but I think that these stems can be analyzed with the method of vital staining.

Naegeli has been the leader of the polyphyletic school since the time of Ehrlich, and I think that it is fair to say that in spite of the brilliance and the dominance of the monophyletic school, the idea first indicated by Naegeli of classifying the white cells of the blood into three groups, leucocytes, monocytes and lymphocytes, has been used continuously in the clinical study of blood, the reason for which now being clear, that this classification is functional.

Clasmatocytes. We are at the present time in the beginning of the experimental phase of hematology. As soon as we pass into this phase it becomes clear that blood, both embryologically and functionally, must be considered as a part of the larger subject of the connective tissues. The beginning of the experimental analysis of the connective tissues was made by the Russian, Maximow, one of the greatest of modern histologists. The first step was in some experiments on rabbits published in 1901-02 in which he introduced two sterile cover-slips under the skin in rabbits and found that leucocytes were the first cells to wander between them; then followed a specific type of cell which he called a clasmatocyte and for nineteen hours only these two cells were present and it was not until later that typical fibroblasts appeared. Thus he showed that in the main there are two distinct strains of cells in the subcutaneous tissue, clasmatocytes and fibroblasts. Four years later he defined the clasmatocyte more specifically by the use of a vital dye, neutral red. If living connective tissue be stained with 1 per cent neutral red, all the cells will be killed and all will stain, but if the dye is used in the strength of 1 to 10,000, and injected into the blood vessels, only the clasmatocytes will react and thus they stand out most clearly. The clasmatocyte is a cell with an oval nucleus that is smaller and stains more intensely than that of a fibroblast. The cell varies in shape, is oval or irregular and has more definite contour than a fibroblast. Its cytoplasm contains granules that stain with neutral red, and mitochondria, and its most distinctive characteristic is certain vacuoles, which are really fluid parts of the cytoplasm which also stain with neutral red.

The next step in our knowledge of the clasmatocyte came in the work of Bouffard (4) in 1906 and was a by-product, as it were, of experiments in connection with the treatment of trypanosomiasis by the injection of benzidene dyes. Ehrlich had injected trypan red hypodermically and had noted that the animals became stained. Following this work and that of Mesnil, Bouffard injected "Toluidin and acid H," and found that the dye was taken into the blood-stream from which it quickly disappeared by being taken up by the Kupffer cells of the liver, the cells of the renal tubules and by certain interstitial cells (clasmatocytes) of organs. Three years later Goldmann (22), directly through the influence of Ehrlich, took up the method and really established it as a method for studying phagocytosis. He first used pyrrol blue and later the less toxic trypan blue and gave a very searching analysis of the reactions of the various types of the cells of the body to these dyes. He brought out sharply that there is a specific cell of the connective tissues, which he called the pyrrol cell, that can be clearly separated from other cells by this experiment.

The method was then used by Evans and Schulemann (15), (16), (17) and a very large group of workers, with the result that the clasmatocyte can now be very accurately defined. Evans and Schulemann proved that the azo dyes do not stain any part of the cytoplasm but that the dye is taken into the cell as particulate matter of specific size and that these granules are segregated and clumped in vacuoles. Neutral red, used as a vital dye, that is, in specific dilutions, stains both preformed granules in these cells and the fluid in the vacuoles. These vacuoles are an organ of the cell for segregating particulate matter which has been called the segregation apparatus by Evans who gives all their synonyms, plasmasomes by Ferrata, the grains de ségrégation by Renaut, vacuoles à grains de ségrégation by Dubrueil and purpur granula by Hammer, dye sphere formations by Rosin and Bibergiel, and more simply the vacuoles by Lewis and Lewis.

In an extensive monograph of Evans and Scott (18), which is now in press in the Contributions to Embryology of the Carnegie Institution of Washington, is given a most complete and beautiful description of the reaction of the clasmatocytes on the one side and the fibroblasts on the other to about 200 of the known vital azo dyes in both acute and chronic stages. Both clasmatocytes and fibroblasts take up the dye as minute particles, the clasmatocytes in greater amount, and the clasmatocytes have vastly greater power of clumping the particles into larger masses and segregating these masses in vacuoles. There the substance is either retained in amorphous clumps or, as Evans and Scott have shown, may form beautiful crystals. They find that though in very chronic condition the amount of the dye in fibroblasts may so increase as even to exceed that of the clasmatocytes and that though fibroblasts develop an extensive segregating apparatus, nevertheless even in these extreme conditions the cells do not become identical and can be separated by the fact that the clasmatocyte gives up the dye much sooner. This work will live as the standard for the complete differentiation of these two types of cells. Every student of histology and pathology should see the clasmatocyte after staining in vivo with trypan blue and with the supra vital staining of neutral red. In either experiment a bit of the omentum or a little of the connective tissue around the pancreas gives a most beautiful demonstration. The differences between the clasmatocytes and the fibroblasts are also most clearly brought out in the method of tissue culture, as shown by Lewis and Lewis, for against the delicate films of fibroblasts spread out in one plane in their preparations, the clasmatocytes show with great clearness.

It is obvious that phagocytosis is a very general property of cells and the original experiments of Goldmann gave a survey of almost all of the types of cells that take up these benzidine dyes which are introduced in the form of particulate matter. The question of the reaction of leucocytes to these dyes has been an interesting one. It is well known that leucocytes are phagocytic, indeed vitally stained with neutral red many of them can be shown to have a stainable vacuole but they are found stained only occasionally after injections of these dyes. Downey (10) showed that they were stained if taken from an occluded vein in which there was dye in the plasma or if taken from the tissues soon after a subcutaneous injection. The question of the reaction of all of the types of the cells of the blood to phagocytosis must be taken up in the light of the fact that it has been conclusively shown that foreign particles remain in the blood stream but a short time. Drinker (11) has recently tested this by using a substance, manganese dioxide, which can be recovered chemically and has followed the fate of a given dose in the body. He has found that in most experiments all of the particles have disappeared from the blood in eighteen minutes, and that at the end of one hour 90 per cent of the material can be recovered from the liver, the spleen and the lungs. The question is therefore one of the relative power of cells in phagocytosis, and there are special cells in these three organs which possess this power in the highest degree. These special cells are all endothelial cells. It is obvious that all of the dye is not permanently stored in these special endothelial cells and the clasmatocyte thus stands out as a cell for storing particles over long periods of time. But in the light of these experiments a negative finding in connection with the blood cells does not prove that they cannot take up the dye since in any experiment the dye is in the blood stream so short a time.

The clasmatocyte has now been most effectively discriminated from the mesothelial or serosal cell lining the pleural and peritoneal cavities in some experimental studies of Cunningham (5 a). This relationship has been the subject of very extensive studies, notably by Pappenheim, Weidenreich and Schott. Cunningham has been able to analyze the reactions of serosal cells to various types of stimuli and has found that their reactions are entirely different from those of the clasmatocytes, the fundamental physiology of the former being differentiated toward secretion while that of the latter is toward phagocytosis. In fact he has shown that the serosal cell is more like a fibroblast than it is like a clasmatocyte. The serosal cell may take up particles of dye, it may become free, but it does not become a clasmatocyte. It is clear that modern histological studies depend on the discovery of such specific criteria for cells as to enable one to distinguish them from all other cells moreover these criteria are often the keys for analyzing their functions. A general survey of cells with reference to these specific criteria is being made in the beautiful studies of living tissues by the method of tissue culture in the work of W. H. and M. R. Lewis.

The differentiation of the clasmatocyte from all the other types of the cells of the connective tissues is the first step in the newer work in hematology. The clearest presentation of the idea of the relationship of the clasmatocyte to the monocytes of the blood we owe to Aschoff and Kiyono. They studied the reactions of cells after the injection of lithium carmine into the circulation, in fact, they say that this procedure was first introduced by Heidenhein. In these experiments they found that the white cells which occur in the thoracic duct, that is to say, the lymphocytes, did not take up the granules of the dye but that there was a group of blood cells especially numerous in three places, the veins of the spleen, the vessels of the liver and the veins of the extremities, which did take up the dye exactly as do the clasmatocytes in the experiments of Bouffard and Goldmann. The fact that not all of the blood cells of one type take up such particles in any one experiment need no longer be a confusing factor in the light of the speed with which such particles are taken out of the circulation. Aschoff and Kiyono regarded these cells as of endothelial origin, and like the clasmatocyte of Maximow or the adventitial cell of Marchand. They called them histiocytes or endothelial leucocytes. They therefore stressed the division of the white cells of the blood into three types, leucocytes, lymphocytes and histiocytes or monocytes. At the meeting of the American Association of Anatomists in 1921, Simpson described the vital staining of films of human blood by the Pappenheim method in which she showed that the transitional cell of the blood stream has a vacuole which is identical with the segregating apparatus of the clasmatocyte. In some studies on the differentiation of blood cells as seen in the living chick (1921) I have been able to prove that the clasmatocyte of the connective tissues and the monocyte of blood are identical cells with an identical origin. Both of them can be seen to differentiate and become free from the endothelium of capillaries and veins in a living form. Ferrata's view that the clasmatocyte may be considered as a stem cell does not seem to me justified because both cells are completely differentiated when they break off from the parent endothelium.

The erythroblasts, the first blood cells. In giving an account of these experiments with a living form one should begin with the erythroblasts since they are the first blood cell to form. Moreover since erythroblasts come from angioblasts and endothelium one must begin with the subject of the origin of the angioblast.

In 1920 the writer (61) published an account of a study of the origin of the vascular system as it can be made out in the living blastoderm of a chick of the second day of incubation. During the second day of incubation the essential processes of the origin of the vascular system can be made out in the area pellucida with very great clearness. Blood vessels start by the differentiation of a new type of cell, the angioblast, from the primitive mesoderm. This cell develops a denser cytoplasm than the primitive mesoderm, due to the fine azur granulation so that it is identical with the stem cell of the monophyletic school. The size of these granules is such as to give the cell the appearance of ground glass. During the second day of incubation all of these basophilic cells can be identified as angioblasts because, as seen in the living form, they remain together after their first division to make syncytial masses. These masses of cells then put out very characteristic sprouts by which they join similar masses to form a plexus. These solid masses of cells become blood vessels by a true cytolysis or liquefaction of the center of the mass to form blood plasma while the cells on the edge differentiate into endothelium. The essential characteristics of angioblasts are the dense, basophilic cytoplasm, the tendency to form syncytial masses, the sprouting to form plexuses, and the liquefaction to form blood plasma. It should be made very clear that there is a tissue fluid before there are any angioblasts, which in the chick is taken in from the yolk by endodermal cells, and that the blood vessels produce their own fluid so that endothelium from the start is a membrane between two fluids, a tissue fluid and a plasma, and the plasma is made from a solution of cells, the nuclei being involved as well as the cytoplasm. It may thus be said that the fundamental morphology of the vascular system has been established.

The next point of importance in the study of these living chicks is that the origin of red blood cells both from primitive angioblasts and from the cell division of endothelium can be proved beyond question. It can be seen in the living chick that when liquefaction is going on, a part of the solid mass may remain and develop hemoglobin as can be made out by the cells becoming yellow or if the cytoplasm of the center of the mass has entirely liquefied the endothelium may divide and give two rows of cells, the inner one of which protrudes into the lumen and will develop hemoglobin while it is yet attached to the wall and divide to make a mass of red blood cells. Thus by watching the living chick of the second day it can be seen that all the primitive blood cells become erythroblasts and that their genealogy is angioblast directly to erythroblast, or angioblast, endothelium, erythroblast. Thus the postulation of a lymphocyte as a stem cell is here unnecessary.

During the present year I (62) have been making further studies of the development of the blood, and have found that the older chicks can be studied in the same manner, the processes being watched under an oil lens. It has been found to be a very great advantage to study these blastoderms with a vital dye, and neutral red is the most convenient. The embryo is studied in Locke-Lewis solution, to which has been added 2 to 3 drops of a 1 per cent aqueous solution of neutral red, a dilution of approximately 1:10,000. From each blastoderm I first draw out one or two drops of blood for films, which are studied with vital dyes and then stained with Wright's eosin-methylene blue. The blastoderm is then mounted and watched for about four hours. The technique for the blood films with a vital dye is that of Pappenheim, to make a very thin film of the dye, neutral red or brilliant cresyl blue, by drawing a clean rod dipped in a saturated alcoholic solution of the dye across a clean slide, and then mounting a drop of fresh blood on this dry film of stain. The specimen is at once ringed with salvoline and placed in a warm box.

In these studies it has been possible to establish a specific criterion for a primitive red cell. At the stage of the second and third day, of incubation, all of the red cells have a very characteristic massive granulation stainable vitally in neutral red and brilliant cresyl blue. It is in the form of granules and rods which make a dense rosette or wreath that completely surrounds the nucleus. It takes a stronger solution of the dye to bring out this granulation than to stain the granules and vacuoles of the clasmatocytes. In the weaker solution the granulation of the red cells becomes just visible though it is not stained. In the living cell the granulation has the same index of refraction as the rest of the cytoplasm and is therefore not to be distinguished. It is stainable however in a dilution that does not stain the nucleus. The granulation is soluble in all of the fixatives, in Helly's solution, in alcohol, in formalin and in the vapor of formalin but if it be stained in brilliant cresyl blue it becomes insoluble in methyl alcohol and so the cells can be counterstained. In the early stages of the red cell or megaloblast the granulation is massive and completely fills the cell; then as the cytoplasm increases the granulation remains in a rosette or wreath around the nucleus making the erythroblast; a little later it begins to spread out or thin out in the cytoplasm making extensive reticular forms. These reticular forms or erythroblasts begin to appear on the third day and they show both the basophilic cytoplasm and the specific granulation. By the seventh day the majority of the red cells are in the reticular form, but the primitive stage and the rosettes are still present. The primitive red blood cells are fairly uniform in size, or at least on the second day one can make out but a few different generations in them, but by the fifth day there is a remarkable variation with very small actively dividing cells, many of them irregular or spindle shaped and much larger, older forms. The smaller forms have the granules in the rosette form, the larger one in the diffuse reticular form. In both of these stages there is polychromasia. Gradually the amount of the granulation becomes very much reduced. The intermediate stages between the seventh day of incubation and the time of hatching I have not yet followed through, but at the time of hatching and for the first three days afterward, all of the red cells have a few granules stainable with brilliant cresyl blue. Some of the cells have only one or two, others up to eight or ten granules while a few of the cells have more. In this stage there is no longer any diffuse basophilia.

This substance I believe to have very great significance in the study of blood. It forms a specific criterion for recognizing the primitive red cell in distinction to other types of the blood cells. The method of embryology gives a chance to follow the stages in its development with exactness, so that one can know how primitive a given cell is by the amount and the arrangement of these granules. Since it is so fundamental, it must necessarily appear in the blood in conditions when blood is regenerating. Besides this, it may offer a chance to study the development of hemoglobin, for example, in testing whether these granules contain iron or not.

The use of vital dyes for the staining of granules in blood cells was suggested by Pappenheim as early as 1894, recommending brilliant cresyl blue at that time, while in 1896 Isreal and Pappenheim (26) showed that if a few grains of neutral red were placed on a slide and used for a blood film, a few granules in the red cells would stain as the cells began to die. The use of neutral red for the blood was introduced by Ehrlich. The subject of vitally stained granules in the red cells was not brought into prominence until 1907, when Cesaris-Demel, Pappenheim and Ferrata all published articles on the subject in the Folia Haematologica. Cesaris-Demel describes and illustrates the substance both in the granular and in the filamentous form, showing differences in the reaction to the stain of these two forms. He discusses the occurrence of cells containing these granules in cases of anemias. The question which comes up first in connection with this vitally stainable substance is its relation to the so-called "punctate basophilia." Both Pappenheim and Ferrata agree that the two substances are entirely different. Basophilic punctation, or Grawitz granules, consists of fine particles of basophilic substance, stainable after fixation, in azur, exactly like the finely granular basophilic cytoplasm. Ferrata (20) believes that these granules, basophilic punctation, that is to say, granules staining in azur, arise as an abnormal clumping "conglobation" of the primitive basophilic substance. On his plate VIII (20) he shows the changes in the basophilic substance in experimental lead poisoning, the earlier phase being a clumping of the basophilic substance in the periphery of the cell, giving patterns very different from those of the vitally stainable substance. This view of Ferrata, that the basophilic punctation is an abnormal change in a young cell, explains why this granulation does not appear in the normal development of blood. That is to say, basophilic punctation would then represent a young cell which has suffered abnormal changes, while the reticular cells are normal young cells which may appear in the circulation under abnormal conditions. The granules which stain vitally I believe to have great significance as being a fundamental substance in evolution of the erythrocyte, which would make clear their appearance in the cells of the circulating blood under conditions of regeneration just as nucleated forms appear in regenerating human blood. When the exact stages of the development of the vitally stainable granules are worked out in the human embryo, as can now be done, it will be possible to estimate just how primitive are the young cells that get into the circulation during the phase of regeneration.

In these studies in the chick four different kinds of stainable substances have been found in the cytoplasm of the erythroblasts. First, the very finely granular, basophilic cytoplasm, staining in azure and appearing like ground glass. This substance is not distinctive because it is characteristic of the primitive type of all blood cells. It disappears as hemoglobin develops. When both substances are stained in the same cell, we have the phenomenon of diffuse basophilia, or polychromasia or polychromatophilia. Secondly, a specific granulation characteristic of the erythroblast, a criterion to separate it from other cells, a substance which is exceedingly soluble but can be made insoluble by combination with certain vital stains, notably neutral red and brilliant cresyl blue. When so stained it is retained in methyl alcohol so that the cells can be counterstained in azur-eosin. It is in the form of granules and rods, which are arranged in characteristic rosettes or wreaths around the nuclei; later the substance becomes less dense and then occurs in a reticular form, and finally, in stages after all of the basophilic cytoplasm has disappeared, is in the form of a few granules or droplets, in the acidophilic hemoglobin-bearing cytoplasm. Thus it persists longer than the basophilic cytoplasm. Thirdly, hemoglobin. In the primitive cell there is the basophilic cytoplasm and the specific granulation. Just the moment of the beginning of hemoglobin is hard to make out, but it can be recognized in a living cell before it can be fixed and stained. It occurs in the cells on the second day of incubation when the specific granulation covers the entire area of the cell. When the hemoglobin can first be stained the azurophilic cytoplasm predominates as has been shown by Pappenheim, Maximow and Danchakoff. The second stage of the red cell has polychromasia and the specific granulation in a dense rosette around the nucleus, the third stage has still polychromasia and a reticulation of the vitally stained granules, and finally the basophilia disappears while the hemoglobin and a remnant of the specific granules are left. This stage characterizes the cells at the time of hatching in the chick.

Fourth, there are the so-called Howell-Jolly bodies. These are fragments of nuclei which were discovered by Howell (25) in 1890 in erythrocytes of the cat after hemorrhage. It is very interesting to note that cells with fragmented nuclei, giving these bodies, occur as early as the second day in the chick and are to be found in almost all specimens. They are to be interpreted as dying cells. They show that there is a certain amount of cell-death even in very early stages of development.

Besides these four substances which are to be found in the course of normal development in the chick, there are two other stainable substances found in the red cells in clinical work which have not been found in the chick. They are the basophilic punctation already discussed and the ring bodies of Cabot, especially well illustrated by Ferrata. The latter are thought to be the occasional remnants of the nuclear membrane, after extrusion of the nucleus. It is clear that an accurate knowledge of the maturation stages of the red cells, or the steps by which a primitive red cell becomes an erythrocyte, is of very great value in estimating the severity of an anemia or in following the stages of recovery. The method of studying the development of the blood by taking it out from a chick on each succeeding day of incubation and using the technique which combines vital dyes with the eosin-azur blood stains enables one to follow the stages step by step as cannot be done in bone marrow. In the chick a specific new stage is added with a given increase in the age of the chick. Thus one can have a specimen of red cells in which there are only the primitive types, and then are added the rosettes or wreaths of the specific granule, then the reticular forms in cells which show polychromasia and finally comes the stage of remnants of the specific granulation in erythrocytes with acidophilic hemoglobin. At the time of hatching all of the red cells in the circulation of the chick show a few of these vitally stainable droplets, some of the cells having 1 or 2, others 8 or 10. The same progression in the development of blood can be followed by this method in mammalian forms, but so far only a beginning has been made.

That endothelium may give rise to red cells was discovered by Danchakoff (6) and confirmed by Maximow (35), Minot (42), Emmel (12), (13) and Jordan (27), (28). The extent of the origin of the red cells from endothelium in the chick was not made clear until studies were made in the living form. The significance of the origin of erythroblasts from endothelium in the chick will, I think, be much less if Maximow's view is finally confirmed that the origin of red cells from endothelium is only transitory and that the secondary, permanent red cells are extra-vascular in origin. The question has two phases, are the cells actually extra-vascular, and if so are they related to endothelium?

Origin of monocytes and clasmatocytes. On the third day the white cells of the blood begin and the origin of two groups can be seen in the area pellucida, namely, the monocytes and the granulocytes (62). By the term monocyte I mean the large mononuclear forms and the transitionals of Ehrlich, grouped together in the third row in Pappenheim's plate 1, (56) and the fourth and fifth rows on Ferrata's plate XII (20).

Of the monocytes, it is the transitional forms that are the easiest to make out because they are identical with the clasmatocytes. On the third day the endothelium of the capillaries and veins of the area pellucida becomes reduplicated in places. All of the endothelial cells have granules that stain in neutral red, scattered throughout the cell. A single cell of the endothelium of the inner row enlarges, protrudes into the lumen, develops the vacuoles that are characteristic of clasmatocytes, and may even engulf a red blood cell. This I have seen in the living chick and such a cell is shown in Maximow's figure 4 on plate XVIII (35). The free border of the cell then develops an appearance characteristic of clasmatocytes, a film of cytoplasm in which a central process is more refractile than the rest, and these films of cytoplasm are in constant motion. These cells I have seen become free from the wall making the transitionals of the blood. Maximow has seen these cells in the vessels of mammalian embryos and has shown three of them in the same figure but did not regard them as a blood-cell type. They are identical with the clasmatocyte and with the transitional forms of adult blood. At the same time the endothelium gives rise to clasmatocytes; the outer cells of a reduplicated endothelium divide rapidly and make irregular patches of cells along the vessels which differentiate vacuoles, many of them very large, and these cells become free and have all of the characteristics of clasmatocytes. The extra-vascular ones tend to be larger than those within the vessels but I have seen one of the large forms pass into a capillary. Thus the transitional cell of the blood and the clasmatocytes are identical forms and both develop from endothelium. Moreover they become differentiated into the specific cell type before they are detached from the wall of the vessel.

The large mononuclear form comes also from the endothelium though this is less easy to demonstrate on the third day because there are so few and because one lacks the characteristic neutral red reaction to pick them out. A special endothelial cell may enlarge and become free with neither the neutral red rosette of the erythrocyte nor the vacuoles of the clasmatocyte. The cells become larger than the others and their nuclei become eccentric. They are most readily demonstrated in Wright's stain after the vital dye brilliant cresyl blue because they lack the characteristic granulation of the red, and because their cytoplasm stains a distinctive clear blue. Thus in the yolk sac of the living chick of the second and third days of incubation it can be proved that endothelium gives rise to the erythroblasts, to the monocyte stem of the white cells and to the clasmatocytes of the connective tissue. On the second day almost all of the cells become erythroblasts, though I have found a few monocytes within the vessels in an occasional specimen. In other words, it may be said that the clasmatocytes are from endothelium, most of them are extra-vascular, a few intra-vascular.

Having proved that the monocytes of the blood and that the clasmatocytes of the connective tissue are derived in the embryo from endothelium and are identical cells we may now go a little deeper into the subject of the clasmatocyte. Maximow distinguished between the resting wandering cell and the active one in his first experimental procedure, indicating that the mononuclear cell of the connective tissues, well known to the pathologist, became transformed by irritation into the actively phagocytic clasmatocyte. The same double group of cells is to be found among the monocytes of the blood, in the large mononuclear and the transitional forms. Both come from endothelium, and it is a question whether the mononuclear becomes in the blood stream a typical transitional or not; in Pappenheim's row of monocytes (56, plate I), the second cell is an undoubted large mononuclear but has a vacuole in which is a remnant of a phagocytized red cell. The large mononuclears of the blood stream may have the relation to the transitional that Maximow sees in the mononuclear cell of the connective tissues to the clasmatocyte, namely, a resting and an active phase of the same cell, or the mononuclears of the blood stream may be for the most part old cells, which would not give rise to typical transitionals. Maximow called the whole phagocytic group of cells of the connective tissue, polyblasts, clasmatocytes, or resting and active wandering cells. He believed that they arose from blood cells (lymphocytes) which had wandered out of the vessels. Marchand (34) then took up the subject and carried it a step farther showing that the clasmatocyte is not a cell which has emigrated from the blood vessels, but is rather a true extravascular cell, which he called the adventitial cell.

The term clasmatocyte was adopted by Maximow from Ranvier who had given the name to an amphibian cell now known to be different; the word means to fragment and in this sense is not descriptive. It has been called the resting wandering cell and the polyblast by Maximow, the histiocyte by Aschoff-Kiyono, the pyrrol cell by Goldmann, the rhagiocrine cell by Renaut and finally Evans has adopted the term macrophage to include all the cells of the connective tissues that phagocytize trypan blue. It is obvious that the term macrophage is not distinctive for the one type of cell, especially since we can now separate the clasmatocyte so sharply from the serosal cell. Now that this cell-type has become so clear that we know its origin and its specific function, we should have a distinctive and a clear name. We may then offer as a definition of a clasmatocyte or histiocyte, that it is a cell derived from endothelium being very highly specialized toward phagocytosis, and possessing a marked development of vacuoles in which particulate matter is segregated, agglutinated and stored and having a differentiation of the periphery of the cytoplasm into films that keep the cell in motion, while the cell itself has only a slow locomotion.

With this definition in mind it will be interesting to take up the work of Mallory and his school on the "endothelial leucocyte," which brings us to the question as to whether clasmatocytes differentiate in the adult. In 1898 Mallory (33) described the reaction of lymph glands to typhoid infection. He first took up the reaction of the follicle proper and then the reaction of the sinus. First in regard to the reaction of the follicle he says:

> In the lesions to be described, however, the large cells of the reticulum of the lymphoid tissue behave in all respects like the endothelial cells of the lymph and blood-vessels. This fact might be used as an argument, therefore, in favor of their being endothelial rather than connective tissue cells. In the following pages these cells will be spoken of as endothelial cells. Under the influence of the toxic product of the typhoid bacilli these larger endothelial cells increase in number.

This indicates that Mallory believed that the reticular cells of the follicle are normally covered with endothelial cells, and he makes this statement still more clearly in his *Pathological Histology* (p. 616). Here it must be said that it is not thought by histologists that in normal lymph glands there is such a spreading of endothelium over the reticular cells of the follicle. The follicle consists of a framework of reticular cells, through which passes a capillary bed; the interstices being filled with lymphocytes. Mall gave us a clear picture of the reticular cell; it is a branched cell of the connective tissue which produces a specific type of fiber, namely, one which is branched to an extreme degree wherein it differs from the unbranched white fibers, but is nevertheless much more closely allied, perhaps identical with the white fiber chemically. The reticular cell differs from the fibroblast which produces the white fibers and the yellow elastic fibers in being more primitive, namely, in that the reticular fibrils remain within the cytoplasm of the cell throughout life. Since in the normal follicle there are no endothelial cells on the reticulum, it may well be questioned whether the "endothelioid cells" occurring pathologically in the follicles have arisen in situ from reticulum, instead of being clasmatocytes which have wandered in.

The structure of the lymphatic sinus and the splenic pulp does, on the other hand, bring out a much more complicated relation between endothelium and reticulum. While in the follicle there is normally one cell, in the sinus there are two. That cells of the monocyte type become free in the sinuses is without doubt, and the question is, can their origin be analyzed, can it be shown that they are endothelium or reticulum? Embryologically the sinus is a zone where there is a plexus of lymphatic capillaries lined by endothelium with bridges of reticulum between, or as in the spleen, a plexus of vascular capillaries with reticulum. In the development of the sinus, the lymphatic plexus or the vascular capillary plexus has become so extraordinarily dense, that the bridges between adjacent vessels are reduced to single branched reticular cells (59). In other words, endothelium is spread over reticulum. Such being the development of the sinus, the question comes up as to whether these two types of cells, which are present at first, can be discriminated throughout life, and this question was ably discussed by Downey at the meeting of the American Association of Anatomists in 1915. In discussing the sinuses Downey (8) said:

> Even with ordinary methods it is evident that the strands of the reticulum are composed of branched, anastomosing cells which are closely associated with the fibers. Nothing can be seen of a continuous epithelial covering. Associated with these strands, especially where the reticulum forms the wall of a sinus, are varying numbers of larger and more rounded protoplasmic cells whose connection with the fibers of the reticulum is not so evident with ordinary methods. Such cells, especially where they project out into the lumen of a sinus, might well be mistaken for hypertrophied endothelial cells. However, the use of any one of the numerous specific stains for reticular fibers (Krause's iodo-iodide of potassium—gold chloride method, the Maresch-Bielschowsky, or the older formula of Mallory's hematoxylin as used by Thomé) shows clearly that these cells are

> frequently traversed by fibers, and that even the large rounded cells resembling large mononuclear leucocytes are frequently attached to the reticulum and have fibers embedded in their peripheral portions. These latter cells show great phagocytic activity, especially for red corpuscles, and their nuclei are large and indented. If these cells were not attached we should not hesitate to pronounce them as large mononuclear leucocytes.

Thus Downey recognized both types of cells clearly but regards them as identical on the basis that both of them contain fibrils. In this connection it is interesting to remember that Mall demonstrated that endothelium can produce reticulum, as in the liver, while Corner has shown that this is true also in the corpus luteum of the ovary. Thus it may be questioned as to whether the presence of reticular fibers is to be considered as an adequate separation of endothelium from reticulum. The question is significant in connection with such a specific development of the power of phagocytosis as has been demonstrated as a characteristic of endothelium and its derivatives. It may be accepted that these two cells cannot be distinguished in sections of sinuses by histological methods. The question then comes up as to whether these cells can be separated by experimental methods. All of the experiments with the injections of particulate matter have brought out that there are four special places where certain cells always phagocytize considerable amounts of any injected particles. These places are the capillaries of the liver and the veins of the splenic pulp, in both of which areas the cells are unquestionably endothelium. The other two places are the lymphatic sinuses and the sinuses of splenic pulp and in both of these areas the question is complicated by the relation of endothelium to reticulum. McJunken (40) has recently made some experiments with the injection of carbon particles in the form of India ink. He analyzed first the nature of the suspension of carbon particles, reducing the size of the particles by filtration. He found that if filtered India ink be mixed with sodium citrate, the polymorphonuclear neutrophilic leucocytes are inhibited from ingesting the carbon and thus the endothelium of the vascular capillaries and the monocytes of the blood can be brought out. By passing the same solution through lymph glands by interstitial injections, he then found that both the endothelium of the sinuses and the monocytes coming from them could be distinguished from the reticulum, while in the follicle proper, only the vascular capillary endothelium showed the carbon particles until stained clasmatocytes had wandered in. He thus separated the lymphatic endothelium of the sinuses from reticulum, and the clasmatocytes of the follicles from lymphocytes. Kiyono (29), on the other hand, finds that with injections of other particles, for example, carmine, both the reticulum and the endothelium of the sinuses take up the particles but that the endothelium can be distinguished by the fact that it always takes up a larger amount. This is more in line with the general experience in these recent experiments in phagocytosis, namely, that the property of phagocytosis is a very general characteristic of cells, but that some strains are more highly differentiated along this line of taking up particles than others. Clasmatocytes take up and store particles to a very marked degree. In the case of the endothelium making the wall of a vessel, it must always be considered whether the granules are passing through the cells or are being stored by them. At any rate, the process of selective phagocytosis does offer a method of showing that the embryonic separation of the two types of cells in the sinuses is retained in the adult, the reticular cells making a framework, the endothelium giving rise to monocytes. Kiyono however believes that both the reticular cells and the endothelium give rise to monocytes (histiocytes).

Foot (21), using India ink as a specific granule to bring out endothelium and the cells that are derived from it, finds clumps of cells on the surface of the capillaries of the lung under the irritation of tuberculosis, which stand out by their ingestion of carbon particles. From this he concludes that clasmatocytes differentiate from endothelium in the adult. If it can be conclusively proved for the adult as it has been proved for the embryo, that endothelium keeps on giving off clasmatocytes in certain areas or can be made to do so by certain stimuli, it will be of great significance in pathology. McJunkin's experiment has the further significance of showing that the endothelium of the lymphatic sinuses reacts like vascular endothelium which is in line with the theory that the lymphatic system is a part of the venous system, its lining cells having the same fundamental origin from the angioblast stem as a differentiated cell, rather than coming from the flattening out of mesenchyme cells to line spaces.

Thus the studies of Mallory and his school on the "endothelial leucocyte" which could now better be called the "endothelial monocyte" all center around the very important question as to whether new clasmatocytes differentiate from endothelium in the adult under normal conditions or can be made to by abnormal stimuli. It seems to me likely that this will ultimately be proved conclusively.

That endothelium in the adult gives rise to the monocytes of the blood stream in certain places seems adequately proved. Thus monocytes can be found so readily and in such numbers in the veins of the splenic pulp that monocytes have been called splenocytes (56, plate I). McJunkin and Kiyono have shown that the sinuses, both lymphatic and vascular, also contribute, and Aschoff thinks that the large number of monocytes found in the veins of the extremities shows that the same cells get free in bone marrow.

It will now be interesting to sum up the history and to show the very wide range of studies which have been necessary to bring out the great group of the histiocytes and to show that the monocytes of the blood and the clasmatocytes are identical cells, histiocytes. The cell-type was first brought out by Maximow by its specific response to injury and then by its reaction to vital neutral red; then the studies of Bouffard, Goldmann, Evans and Schulemann and many others have brought out the fact of the power of these cells to phagocytize, segregate and store particulate matter. Evans then made the final differentiation of the clasmatocyte from the fibroblast, while Cunningham has conclusively separated it from the serosal cell. The next step came in the doctrine of the origin of blood cells from endothelium by Mallory, by Schridde (64) and Schmidt (63). Then Aschoff and Kiyono showed that the monocyte in the blood stream has an identical reaction toward particulate matter (65), that the reaction to neutral red was the same. Aschoff, Kiyono, McJunkin and others have shown that the endothelium of the adult both lymphatic and vascular can in certain specific places give off monocytes, and now it has been conclusively proved embryologically that the type of the white cell that comes from endothelium is the monocyte, which is identical with the clasmatocyte. Both cells have been seen by the writer in a living embryonic form to differentiate from endothelium and to become free, the monocytes dropping off within the vessels, the clasmatocytes outside; but the cells are interchangeable afterwards, for the clasmatocytes have been seen to enter the vessel and the monocytes may pass out. The entire group has a very characteristic type of motion due to a differentiation of the periphery of the cell which keeps the surrounding fluid in motion while the cell itself has but a very slow grade of locomotion.

Origin of granulocyte. The origin of the granulocytes can also be made out on the third day of incubation. As shown by Danchakoff (6) the granulocyte, the forerunner of the leucocytes, is always an extra-vascular cell. It appears from the mesoderm and lies near a vessel and at first cannot be told from a single angioblast. It has the same dense azurophilic cytoplasm. As soon as it has once divided however it can be differentiated because the two granulocytes separate, while two angioblasts remain together. In the living specimen or in the fixed blastoderm this can be made out as it cannot be made out in sections because in the total blastoderm every cell of an area can be seen which gives this type of material an enormous advantage over sections. The nucleus then becomes eccentric and the centrosome is made very obvious by the development of a crescent of very fine uniform granules, also stainable in very dilute neutral red. These cells then move toward the vessel, often line up in a row in order to enter. They enter the vessels half way between the nuclei of the endothelial cells which means that they push their way between the cells. They make the wall bulge in and once I have seen the edges of the endothelial cells separate. They move very slowly, but it is a real locomotion, not the movement in one place of the clasmatocyte. There is no active amoeboid motion of these cells at this stage, meaning a typical amoeboid locomotion associated with a fluid state of the cytoplasm as shown by active flowing and streaming of granules within the cell. During the third day the granules of these cells are always still and always in a definite crescent around the centrosome. Thus the granules of the endothelial cell that stain with neutral red are larger and are scattered throughout the cell, the granules of the erythroblasts are arranged around the nucleus while the granules of the young granulocytes are arranged around the centrosome. It may be possible subsequently to show that the substance which stains with neutral red in endothelium is not present in primitive granulocytes. That is, it is possible that a further study with vital dyes may differentiate the original angioblasts from the granulocytes. The early granulocytes are all pseudo-eosinophiles, that is, they have fine granules. The eosinophiles with the granules in the shape of rods, so characteristic of the chick, develop a little later; I have not found any on the third day and but few during the first seven days. There are no cells to be made out with the basophilic granulation or mast cells and Maximow has found that they develop late in mammals.

From these observations it seems it seems fair to say that no cell has as yet been identified as a common stem cell for the angioblast and the granuloblast. Indeed it has been the contribution of Naegeli throughout to show that there is no adequate proof that the primitive form of each type of blood cell is to be identified as a common stem for all. The only proof which would be adequate of a common hemoblastic stem would be an experiment in which the stem cells in an area where they all become angioblasts normally, should be made to become granulocytes, as for example if the single basophilic cells of the area pellucida of the chick of the second day which normally make syncytia of angioblasts could be forced to make granulocytes. This is not beyond the range of possibility as, for example, Murphy (48) found that the injection of splenic tissue into a chick stimulated a very marked formation of blood cells in the allantois and in the diffuse connective tissues of the embryo far beyond the normal limits. Thus a connective tissue of the embryo which does not normally do so can be made to differentiate into blood cells. This experiment he suggested to Madame Danchakoff and she has been utilizing it in recent studies (7).

Weidenreich (67) has given an extensive study of the granulocytes paying especial attention to the forms of the nuclei; he has shown that the three types of granular cells have characteristic nuclei. The neutrophilic leucocytes have nuclei with small lobes, often five in number, while the nuclei of the eosinophilic leucocytes rarely have more than two lobes. The mast leucocyte he has proved to be a dying cell without a centrosome, and with irregularly staining granules. In all of the chick blastoderms there are a few dying blood cells with fragmented nuclei but they are always easy to distinguish.

Lymphocytes. In the study of the living blastoderms up to the seventh day, which is as far as I have yet carried the work, I have not seen any evidence of a formation of the lymphocyte in the yolk sac. Further studies may bring this cell out, or it may be that the lymphocytes form only within the embryo itself. The lymphocyte appears in the circulating blood on the fifth day, in its smallest form; an occasional one may occur on the fourth day. In the living state it has a nucleus with a sharper nuclear membrane than any other cell. This is a difficult criterion to use since all nuclei become distinct as the cells die. It has a small amount of cytoplasm and a few azur granules. Lymphocytes are readily killed by too strong a dose of the dye; the granules do not stain readily in neutral red but may be made to by increasing the amount of the dye. In the stained blood smears, the lymphocytes are easily distinguishable by their characteristic nuclei and the fact that they are all at first small lymphocytes. Their origin in the embryo needs much further study, but from their late appearance as a specific cell type it seems to me that they should not be identified as a stem cell. In the general use of the term "lymphocyte" or "lymphoidocyte" as a stem cell, it has been, however, more commonly the large mononuclear form or monocyte so identified rather than the small lymphocyte.

For the adult, it is well recognized that this cell occurs in follicles in lymph glands and spleen, where it probably comes from the reticular cell, and it is swept into the blood stream in the lymphatic sinuses and the splenic pulp. Thus our knowledge of this cell in the adult stresses that it is a cell of extra-vascular origin. The stem cell of the lymphocytes is far from adequately known. It needs study both in the embryo and in smears of living cells from adult lymph glands.

The lymphocyte has been distinguished as a specific functional group by Murphy and his school (46), (47), (48), (49), (50). The lymphocyte group is more sensitive to x-rays and to radium than other normal cells. With a certain dose it is the first normal cell injured, with a less dose it is stimulated. Murphy has demonstrated a specific relation of lymphocytes to immunity toward tumors in the chick, by showing that before there is any great mass of lymphocytes, that is, on the eighteenth day when they form in large numbers in the spleen, chicks are susceptible to tumors to which hens are immune. Then he has killed the lymphocytes of the hen by x-rays and produced susceptibility to the tumor. These studies are still being carried on by Murphy and are one of the very valuable contributions of modern medicine.

The entire absence of autolytic and peptic ferments in the lymphocytes separates them from both leucocytes and from monocytes.

CONCLUSIONS

In stressing the value of embryology as a part of the newer form of the experimental work on blood we may call attention to this advantage, that each cell as it first differentiates is specific, for example, the erythroblast of the second to the fourth day with its rosette of granulations arranged with reference to the nuclei. The erythroblasts have however a long period of maturation before they become the red blood corpuscles of the adult stage. The clasmatocytes and identical monocytes on the third day have a characteristic segregation apparatus, and peculiar type of motion; the granulocyte on the third day has its specific type of granulation, chemotactic response and characteristic arrangement of the granulation to the centrosome, and finally the small lymphocyte on the fifth day has its peculiar nucleus. That subsequently, when all these types of cells are dividing, it becomes difficult to distinguish all the young cells, the whole history of hematology attests and I doubt if every type of cell in adult bone marrow can be identified before completion of the study of developing blood in the entire cycle of the embryo with vital methods has been accomplished, especially studying the cells just after division.

It is now possible to suggest as a working basis for further study, that there are three strains of cells of the connective tissues which contribute to the blood cells. All of these strains give more cells to the connective tissues than to the blood. They are the angioblasts which give the endothelium, the erythrocytes and the monocytes; of these the red corpuscles are all intra-vascular unless the extra-vascular origin of red cells in bone marrow of mammals proves correct. The red cells have a specific granulation which can be stained vitally and is arranged around the nucleus. The monocyte stem is largely extra-vascular, in small part intra-vascular, giving the histiocytes which are the monocytes of the blood and the clasmatocytes of the connective tissues. They have developed along the line of the phagocytic power of their parent cell, endothelium, and are especially differentiated to take up and store particulate matter. It has, I think, become clear that certain endothelial cells, like the Kupffer cell of the liver, which is a specialized endothelial cell within the capillaries of the liver anchored out into the blood stream by guy ropes of cytoplasm, and the endothelial cells of the veins of the splenic pulp as well as the endothelium of the splenic sinuses have the maximum power of phagocytosis and that they clear the blood stream of foreign particles within a very few minutes. Such foreign particles are constantly excreted by the kidneys but if more particles are introduced than the kidney cells can excrete for the time being, the clasmatocytes of the connective tissues take up the dye, store it and eventually give it back for excretion. It is obvious that the amount of dye in the experimental procedures is very far in excess of any usual amount of foreign material to be found in an organism; moreover the substances used in the experiments are all insoluble. These same cells take up red blood cells and cell débris. How extensive their function and how related to normal metabolism in the taking up of substances which they can dissolve is not known. Shipley (64a) regards their vacuoles as organs of digestion.

The second strain is the granulocyte. These cells, certainly the neutrophilic leucocytes, are also phagocytic but they are developed especially along the line of a true amoeboid type of motion with fluidity of the cytoplasm and marked flowing of the granulations associated with great speed of locomotion. They develop specific types of granules which are at first very definitely arranged in a crescent around the centrosome. They are specifically sensitive to certain chemicals, that is to say, they have a high degree of chemotaxis. The small amount of knowledge we have of these cells seems to verify Ehrlich's theory that their functions are related to their specific granules, since it has been shown that the neutrophilic leucocyte has a proteolytic action probably brought out when the granules are set free from the cell. Thus pus from neutrophilic leucocytes becomes fluid. The neutrophilic leucocytic cell responds to certain known stimuli such as the pyogenic bacteria or their toxins, the eosinophilic to certain others such as those produced by parasites. The mast cells are the least understood and are granulocytes which develop late and remain extra-vascular, since the mast cells of the blood have been shown by Weidenreich to be degenerating cells and not true mast cells. Mast cells occur in the bone marrow, along blood vessels and between muscle fibers; their function is totally unknown.

The third strain is the lymphocyte which is the last to develop and is exceedingly widely distributed. It occurs in lymph glands, hemal glands and spleen, that is, in a reticulum near lymphatic or vascular sinuses by means of which it enters the blood stream. It also occurs in small follicles near lymphatic capillaries and ducts or even in small clumps without such relation very widely distributed throughout the organs and in bone marrow as well. Thus it may occur wherever there is reticulum. In fact it makes the third great stem of the diffuse connective tissue cells, which are clasmatocytes, fibroblasts and small round cells or lymphocytes.

Thus the separation of these three strains of white blood cells by the embryological method seems to correspond with functional groups as far as they are yet vaguely known, the leucocytes being highly amoeboid types, with specialized chemotactic responses and functions probably related to their granulations, the monocyte stem with its specialized powers of phagocytosis and the lymphocytes with their marked sensitivity to x-rays and their relation to certain forms of immunity.

The sharp separation of the blood-forming organs into two groups, bone marrow and lymph glands, as Ehrlich thought, has not been borne out. In the first place, blood is not so sharply separated from the connective tissues in general. In the embryo the origin of blood from the connective tissues is very widespread and can be made much more so by certain experimental stimuli. In the adult the location of the differentiation of blood cells is much more restricted. Two cells are necessary, reticulum and endothelium. The reticular cell in its relation to blood needs much more study. Under normal conditions the origin of the red cells is restricted to the bone marrow, the origin of the histiocytes of the blood takes place certainly from endothelium in certain special areas in the adult. How much the differentiation of new clasmatocytes goes on normally in the adult or how much it can be stimulated, is not yet clear. The origin of granulocytes cannot be regarded as retricted to the bone marrow when one considers the eosinophiles and the still more widely distributed mast cells. Lymphocytes arise in the bone marrow as well as in all so called lymphoid tissue.

The study of the blood at the present time has passed beyond the morphological stage and is a part of the development of modern experimental cytology. In this study the anatomist, the physiologist and the pathologist meet on common ground.

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ARTERIOSCLEROSIS

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The following review of the present status of our knowledge of arteriosclerosis was attempted unwillingly for it must be apparent to all that most of the extensive literature upon this subject consists of reviews. These are based upon the few papers which represent real study of the anatomical and functional changes, so that apart from the conflicting results of these few investigators one is impressed by the feeling that most of what is written has only the value of opinion and that we are extraordinarily ignorant with respect to this common affection. Diametrically opposite views are put forward as to the causes, the anatomical nature and effects of arteriosclerosis, many names of different significance are proposed for it and withal there has been no unanimity of opinion as to what should be included under this heading and what excluded.

For the purposes of this review I shall use the term arteriosclerosis rather than atheroma, endarteritis deformans, atherosclerosis or any of the other terms already proposed, simply because it is the most familiar and best established and because it expresses accurately enough our very inaccurate knowledge of the condition.

For the purposes of this review we must exclude from consideration syphilitic aortitis and arteritis, distinctly infectious processes in the arteries caused by other organisms such as the tubercle bacillus and the unknown cause of periarteritis nodosa, traumatic and toxic injuries leading to thrombosis and healing processes in the vessels, and perhaps with less justification, all the forms of obliterating endarteritis which cause the occlusion of the vessels of the extremities and of organs.

Arteriosclerosis remaining for consideration here is an affection of the arteries whose cause is not yet clearly known. It is characterized by constant anatomical changes of which the most conspicuous is a thickening of the intima accompanied by fat deposits. Changes in the muscular tissue of the media and in the elastic tissue are sometimes extreme but often very inconspicuous. These changes indicate a deterioration in the functional capacity of the walls and are followed by necrosis, calcification, etc., which are secondary changes but which generally constitute the most obvious alteration in the diseased vessel wall.

Marchand sums up arteriosclerosis in its wider sense as all those changes of the arteries which lead to a thickening of the wall, especially of the intima, in whose development degenerative changes (fatty degeneration with its results) sclerosis and calcification (including calcification of the media) arise together with inflammatory and productive processes.

It must be remembered, however, that while all students of arteriosclerosis recognize the occurrence of lesions in the various coats and tissues of the vessel they are far from unanimous in their conceptions of the order in which these arise and of the relations which they bear to one another. For some the lesions of the media are primary, those of the intima secondary or compensatory—for others the reverse is true. Equally discordant opinions are expressed as to the disturbances of function which result from or are the causes of these changes. The time is ripe for a searching new study of the whole matter, but little can be expected from a review.

Nevertheless, the available material may be considered under three heads:

1. The anatomical changes in the vascular system.
2. The associated functional disturbances.
3. Etiology.

1. The anatomical changes in the arteries. The current descriptions of the histological structure of the arteries leave much to be desired (Renault, Kölliker, Ebner, Grünstein, Klotz) but in so far as this concerns the arrangement of the musculature and elastic tissue it is due, as v. Ebner points out, chiefly to the great variability of structure in different parts of the arterial tree. Not only are there constant differences among the arteries but the structure of the wall at the same point in a given artery may vary in different individuals. It is with regard to the minuter structure of the intima, however, that most uncertainty persists.

In general the aorta and those trunks which developed in the branchial arches maintain a lamellar arrangement of the elastic tissue in the media while such arteries as the coeliac axis, the superior and inferior mesenterics, renal arteries, common iliacs and femorals present a media composed essentially of smooth muscle with a cobweb-like arrangement of delicate elastic fibrils which run in all directions, while the denser elastic tissue is relegated to the adventitia. The popliteal and tibial arteries are said to be exceptions to this rule but this is not true, and while the brachial arteries for some distance show lamellated elastic tissue in the media, the more peripheral parts of the arteries of the arm have the structure of those of the leg. This lamellated structure of the media is also found for a short way in the proximal parts of the intercostal arteries and exquisitely developed in the pulmonary artery.

Longitudinal tangential section of such a vessel with lamellated elastica in the media shows very plainly the flat spiral arrangement of the smooth muscle fibers which alternate their direction in successive layers so that in the section they appear to take a herring-bone arrangement. The laminae are supplemented by numerous fibrils of elastic tissue which accompany the muscle fibers. Tangential sections through the media of vessels which have no lamellated elastica show no such rapid alternation in the direction of the muscle fibers. In them (renal) there are sometimes bundles of longitudinal muscle fibers outside the media accompanied by the longitudinal elastic fibers of the adventitia.

The elastic tissue of the adventitia is chiefly represented by longitudinally placed fibrils which take a steep spiral direction but in such vessels as the superior mesenteric and coeliac axis there is generally a quantity of elastic tissue coursing in a circular direction as though pushed out from the media. An external elastic lamella is described in many vessels but it is usually not a distinct lamella but only the last lamella of the media or the innermost elastic layer of the adventitia. The development of adventitia varies greatly in different tracts of the vascular tree. It is important in carrying the vasa vasorum but little attention has been devoted to it otherwise in the consideration of arteriosclerosis. Longitudinal muscle bundles are sometimes a very prominent constituent.

In general the intimal coat has been the center of interest. There is no difference of opinion as to the constant endothelial lining except that some early authors attempted to show the origin of the connective tissue layers from these cells, an idea easily dismissed by Marchand. The tissues outside the endothelial layer and within the media have been discussed by Langhans, Kolliker, Ebner, Eberth, Ranvier, Key Aberg, Jores and many others, and a fair unanimity of opinion arrived at, although much of the earlier work was done in the lack of all the more recent staining methods. Key Aberg described the internal elastic lamina with its associated longitudinal smooth muscle fibers and the innermost layer of a tissue more difficult to interpret. This is composed of a finely fibrillar or granular ground substance in which lie the cells first clearly described by Langhans although pictured also by Virchow. These are much branched cells, sometimes very large and stretching far and wide their long processes which, like the rest of the cell body, often contain granules which are probably fatty. He distended with an asphalt-chloroform injection mass a network of canaliculi which correspond fairly well with the outlines of these cells. Similar canaliculi could be injected in the media but in both cases his drawings remind one of the artificial spaces which can be formed in a similar way in the cornea and scarcely suggest preformed spaces. In several attempts I have so far not succeeded in injecting india ink into any spaces in the thickened intima of a sclerotic aorta but once air was driven into the tissue so as to distend a network of tiny spaces.

Jores devoted a great deal of space to the consideration of the histology of the intima of the aorta and recognized a musculo-elastic layer (Thoma) composed of a thin outer and a thicker inner layer of elastic tissue between which were the longitudinal muscle fibers. Inside this he describes a fibro-elastic layer in which laminae or fibers of elastic tissue derived from the internal elastica are interspersed with connective tissue elements. Still nearer the lumen is a layer of almost pure connective tissue.

In my own observations the variation in the structure of the intima is so great in different vessels that any single description seems conventional. It is true that at the inner margin of the media one can generally distinguish a musculo-elastic layer which is composed in its most typical form of an outer single smooth lamina of elastic tissue, a layer of longitudinal muscle fibers and an inner layer of elastic tissue, less simple than the outer and clearly made up of a network of fibers or laminae. There must be a chemical difference between these two elastic structures for the outer stains red when the inner fibers are blue, or brownish-red when they are purple. The inner layer is often in immediate contact with the endothelium in smaller vessels. It is not a single lamella as a rule, although it may appear so in places, but tends to split up into a whole series of lamellae or rows of fibers. Jores' emphasis of this as a hyperplastic or hypertrophic process seems labored when it is so generally present and it is so difficult to find any part of any artery in which it forms a single lamella. Other finer elastic fibrils spread away into the innermost layers of the intima. There seems to be no pure connective tissue layer on this account and in any case the differential stains do not bring out much connective tissue in characteristic form in the intima. In a section stained by Van Gieson's method the intima has a vague purplish color in great contrast with the sharp red of the adventitia. The cells are extremely inconspicuous until arteriosclerotic changes arise. Under those conditions they can be described in detail later. Such cells as are seen in the normal intima have the appearance of smooth muscle and are often seen to be continuous with the smooth muscle of the musculoelastic layer. However, this is a point left unsettled by Langhans, Key Aberg and all the others. The tendency is to regard these branching cells as of connective tissue origin although it is impossible to decide on morphological grounds alone. It is, however, evident that the elements of the internal elastic lamella offer no obstacle to the extension or growth of tissue from the underlying media into the region of the intima and it seems unnecessary to ascribe the origin of all tissue found in the thickened intima in arteriosclerosis to the cells of the intima itself. Indeed, cells appear there which could hardly have originated in the intima.

On such a basis of normal anatomy the descriptions of arteriosclerosis have been constructed. It is possible to pass over the early descriptions of Rokitansky and Virchow and even those of Koester, who appears to have described the mesarteritis of syphilis. Nevertheless, we shall have to recur to Koester's ideas of the part played by the vasa vasorum. Marchand, Ribbert, Reich, Thoma and others have opposed him on the ground that arteriosclerosis is not an inflammatory process in the precise sense of the word but rather, as Marchand said, a nutritive disturbance of the vessel wall followed by sclerosis and degeneration.

Thoma has made himself conspicuous by his long campaign in favor of a mechanical origin of the condition. His theories are hardly generally accepted now but there are some adherents. In principle he thinks the whole process dependent upon a localized or more widespread weakening of the muscular media which bulges outward, thus bringing about a slowing of the blood stream in the widened area. This, by some means not clearly explained, leads to a new formation of tissue on the part of the intima, sufficient to fill up level the depression produced by the bulging of the media. When the artery is laid open this patch of fibrous tissue is thrown into relief by the turning outward of the elastic wall—but if the artery be injected with paraffin at the blood pressure, the cast on cooling will show a smooth wall.

Every point in Thoma's argument has been pretty surely demolished by his critics. The weakening of the media is not obvious until a late stage is reached and then it may well be the effect of the dense plaque of tissue lying upon it. There is no cogent reason to believe that a slight dilatation would be compensated by the new formation of tissue. If it were, it ought to be formed as in the case of the granulation tissue, which replaces the stagnant column in a ligated vessel. In that case the mode of development of elastic tissue should be Jores' regenerative type and there is difficulty in understanding the colossal amounts of fat which are lodged in the tissue from the beginning. Thoma begins with the scholastic argument that in the aorta of the embryo there is no connective tissue intima and that it is the closure of the ductus Botalli and the umbilical arteries that brings about a change in pressure in this portion of the circulation which thus becomes too wide and must be adapted by new formation of connective tissue in the intima. This is the same principle which he uses in explaining arteriosclerosis when he claims that a slowing of the stream from local dilatation produces a compensatory thickening and releveling of the wall.

Jores writes at very great length on what is represented as a totally new attitude toward arteriosclerosis which consists in distinguishing at first in smaller and then in larger vessels between two modes of formation of new elastic tissue in the intima. Jores' earlier studies had shown that elastic tissue in the form of very fine fibrils could be formed afresh in new connective tissue. This process he recognizes now in the tissue which arises in the organization of a thrombus in a vessel or in that which brings about the obliteration of a small artery. He names it the regenerative form of connective tissue growth of the intima and contrasts it with another which he calls hyperplastic thickening of the intima. The latter consists in the appearance of many lamellae or filaments of elastic tissue within the internal elastic lamella which he thinks are split off from the original lamella. He admits that much of this is obvious under physiological conditions but that it becomes emphasized and is the predominant character of the pathological thickening of the intima.

Of course, the occurrence of these two forms or states of division of elastic tissue is true but it seems to throw no light on arteriosclerosis to distinguish them, for one is a characteristic of the formation of new actively growing tissue, the other is formed normally and is only made more conspicuous by the separation of the fibrils or lamellae by the increase of other tissue in the arteriosclerotic plaque.

It seems difficult for Jores to bring himself to make the statement that this is an abnormal condition and he contents himself with saying that it is a departure from the physiological state. All the rest of his suggestions about the effects of high blood pressure in augmenting this and the effects of strains which bear upon the longitudinal muscle seems pure speculation. He does, however, recognize the fact that degenerative changes with the accumulation of fat assume a primary place in the development of arteriosclerosis.

Voigts, in his dissertation, set the matter of the distribution of elastic tissue and connective tissue in the intima in a clearer light and showed, as described above, that the elastic fibers inside the musculo-elastic layer are abundant and form no definite lamellae but only networks. Jores had shown that fine fat droplets adhere to the elastic tissue of the intima except the actual internal elastic lamella and also involve the media. This is readily seen in any frozen section from a sclerotic vessel stained with Sudan. A fine and diffuse red brown staining is found in patches in the media and in the intima, but while the wavy lamella which forms the outer limit of the musculo-elastic layer is unaffected, the palisade-like row of fibers which forms its inner limit is finely strewn with red globules. Torhorst thinks this is not a deposition of fat in the elastica itself but rather in the interstitial supporting substance.

Disintegration of the elastica has been described by Manchot and others. Dmitrijeff describes degeneration and disappearance of fibers and new formation of elastic tissue in the course of arteriosclerosis. Matusewicz also describes and illustrates the more isolated calcification of portions of the elastica in the arterial wall and McMeans discusses in great detail the degenerative processes in the elastica associated with splitting and breaking of the fibers.

Such theories as that of Thoma which ascribe the sclerotic changes to localized dilatation through weakening of the media, must refer this weakening to the smooth muscle or to the elastic tissue of that wall but definite observations of alterations in either of these tissues except in late stages of the disease are hardly to be recognized. It is of course true that under thick degenerated sclerotic patches one ordinarily finds the media stretched thin with perfectly straight and rigid looking elastic lamellae and very much attenuated muscle fibers all pressed together into a mass whose details are indistinct. In earlier stages it is possible to find many breaks in the elastic lamellae but it is difficult to avoid the idea that these may be due to artefact in making the section—nevertheless, in many cases on tracing the homogeneous wavy elastic lamella that bounds the musculo-elastic layer toward the media (the true internal elastic lamella) breaks are found which cannot be due to artefact for from the separated blunt ends of the broken lamella there spring delicate elastic fibrils which bridge the gap. Destructive changes in the muscle of the media are rather more definite when they are found in a section in which the elastic tissue is stained for they then appear as patches filled up by many collapsed elastic lamellae between which no traces of muscle remain. The granular degeneration of elastic fibers described by Jores, Weiszman and Neuman, is regarded by Voigts as not an abnormal condition.

Ribbert expresses the idea that inasmuch as some areas of the best arterial wall may be weak and easily bulged out by a high blood pressure, it might readily follow that the corresponding area of intima becomes stretched over this so that its meshes are laid open and soon filled with plasma pressed in from the blood stream. This might account for the hyaline edematous appearance of the plaques thus formed and perhaps also for the advent of fat which is so uniformly present. Nothing in this idea seems plausible for it is difficult to imagine such a power of resistance in the intima, and further difficult to understand why it does not protect the underlying media from the distending force. The imbibition of blood fluid which may well occur could hardly give rise to all the extraordinary new tissue which forms the plaque and which, if we may believe Reich's statements, is not necessarily formed in the dell of an area of stretched out media since he finds the internal elastic lamella fixed in corrugated form between the plaque and the media while the normal elastic lamella stretches out smooth when the artery is distended.

Alb. Aschoff and also L. Aschoff trace in detail the gradual development of the intima with its increasing elastic tissue from early youth to mature age. After this there is a period of quiesence which is then followed by another period of advancing senility in which no more elastic tissue is formed but much new connective tissue. This leads the latter to the view that the loss of elasticity is the basis upon which a compensatory strengthening by the formation of new connective tissue occurs, an idea which is not very different from Thoma's. Klotz, too, in a long and careful paper upon the importance of medial alterations, concludes that sclerosis of the vessels is largely dependent upon disease of the media brought about by infections, poisons, work or old age.

The discussions of the actual anatomical changes in arteriosclerosis have brought forth little that is new. Interest centers especially in the intima although Thoma and his students, Klotz and others, conscientiously search for primary alterations in the media. Aside from the extensive changes in the media of the peripheral vessels which are quickly followed by calcification or bone formation and scarring with changes in the intima which may be comparatively insignificant or which may have the character of an obliterating endarteritis, the media in the aorta and visceral arteries seems relatively little changed in comparison with the extent of the internal alterations. Such changes as there are may be recognized by measurement of the thickness of the media or by the finding of actual degeneration and disintegration in the muscle and elastica beneath the plaque, always suggesting that they may be secondary to the presence of the plaque, as easily as its cause. Little attention has been devoted to the adventitia but Klotz and Adami point out that this coat too is sometimes thickened opposite an arteriosclerotic plaque. I have seen this also but it is so irregular and the tissue so loose that it is hard to believe that it really affords protection to that area.

In the intima Klotz has studied especially the fatty streaks which are so familiar and which he and many others describe as occurring in the same distribution as the earliest arteriosclerotic lesions in the aorta. This I think is not true for the yellow flecks as a rule form an almost continuous line along the dorsal intima of the aorta and circling round the innominate or carotid while the actual sclerotic plaques are quite irregularly placed, often, however, surrounding the orifices of the intercostal arteries. These fatty flecks have been thought by many to be the transient effects of infection or intoxication, disappearing after convalescence. This is of course difficult to prove but it seems plausible. Klotz has described them minutely showing that the fat, some of it anisotropic, is accumulated in cells resembling lutein cells which Anitschkoff calls cholesterin phagocytes, and also in other larger branching connective tissue cells. The greater part is apparently taken up by the cells of the first order which are round cells with abundant protoplasm loaded with oil globules, and a small deeply stained nucleus. They are clustered loosely in clefts in the superficial intimal tissue. The branched cells which contain fat are marked out to their distant branches by the fine globules.

There is no sharp line to divide this earliest condition from later stages in which there is associated with these fat accumulations a considerable new formation of tissue including elastic tissue fibrils. The later anatomical changes seem to consist in further accumulations of fat, in the multiplication of connective tissue cells and to a slight degree of fine elastic fibrils, in the appearance of much new hyaline fibrous tissue with a great deal of material which microscopically has the appearance of coagulated fluid. Necrosis and disintegration of the tissue about the fat occurs and as the fat has by this time spread into the musculo-elastic layer and even into the media these tissues tend to be involved in the disintegration. But below the accumulation of fat and more especially above it, the newly formed tissue becomes dense and pearly so that the fat may not be visible through the plaque thus formed. The later alterations which result from the decomposition of the fats, glycerin and cholesterin esters and lecithids, are well known. Cholesterin crystals appear in the formless mass and calcium is deposited in the form of carbonates and phosphates. Klotz has explained this as the result of the formation of calcium soaps and their later decomposition with the production of calcium phosphates and carbonates but Baldauf and Wells object to this theory on the ground that they can isolate no calcium soaps from sclerotic arteries. It seems a plausible idea, however, and the soap stage must at best be very evanescent.

The dense pearly tissue which forms the plaque over (and under) the necrotic mass (atheromatous material) in which the fat lies is interesting on account of its microscopic characters. It does not give very clearly the reactions for connective tissue with Van Gieson's stain nor with Mallory's aniline blue. The bands of material of which it is composed are hyaline and do not look like connective tissue. There are peculiar long branching tubules with dense refractive walls which with the intervening and differently staining hyaline material make up the mass. In each of these is an elongated cell probably the branching cells described by Langhans and Key Aberg. It seems most reasonable to regard these as connective tissue cells but their nature is by no means certain.

Although Klotz and many others draw a distinction between the arteriosclerosis of the small vessels and that of the aorta, it seems that exactly the same changes are to be found throughout. If they refer to the Mönckeberg type of medial sclerosis, the distinction is plain, but it seems that the arteriosclerotic changes which occur in the aorta are also in most cases to be found in the peripheral vessels.

The associated functional disturbances. Under this heading we should consider the distribution of the diseased condition of the arterial walls, the effects upon the circulation and the heart, and the effects upon the tissues supplied by the altered arteries.

Most authors (Stengel, Brooks, Ortner, Perutz, Goldscheider and many others) lay stress on the localized occurrence of arteriosclerosis in certain areas leading to the production of symptoms which may be ascribed to the condition of the circulation in those vessels. Doubtless if this is true at all it may be true of many organs but emphasis is laid upon the existence of localized sclerosis of the arteries of the brain, of the heart, the kidneys and of the intestinal tract. In the case of the brain, aside from actual hemorrhage from ruptured arteries, transient losses of function are often ascribed to a spasm of the arteries or to temporary inadequacy of the blood supply on account of the narrowing of the arteries. There seems to be no convincing proof of the occurrence of a spasm which might produce such a cessation of function and even when there are obvious encroachments upon the lumen in the arteries the explanation of a temporary lapse is difficult. The whole subject of angina pectoris which is associated with sclerosis of the coronary arteries is too extensive to consider here. There as in the case of the crises of pain which sometimes accompany arteriosclerosis of the mesenteric vessels, opinion varies as to whether pain is due to a cramp of the vessel walls or to the sudden ischemia produced by such narrowing.

With regard to localized arteriosclerosis of the branches of the renal arteries, there is a great literature and recently (Lohlein) even a further localization in the arterioles of the glomeruli is held responsible for many of the disturbances in function. Jores finds that sclerotic changes with hyperplasia of the internal elastica are not present in all cases of contracted kidney in chronic nephritis nor in the acute and subacute forms in which there is already high blood pressure. But this too is a subject which cannot here be considered in detail.

With regard to the effect of general and localized arteriosclerosis upon the circulation there has been much speculation without as much actual observation and experiment.

The arteries become rigid, lose their distensibility and resilience, lose to some extent their muscular contractility and are in addition in some cases dilated and deformed, in others intermittently or extensively encroached upon by thickenings of their walls. One may frequently observe this last fact in the coronary or mesenteric arteries in which, while much of the artery is thin walled and dilated, there are marked constrictions at certain points produced by thick projecting plaques of sclerosis. These are, in general, affection of the larger and medium-sized vessels. The very small arterioles and of course the capillaries are in general less affected although in some localities, as in the kidneys, they are definitely thickened and narrowed.

The effect of these changes upon the general arterial blood pressure is one point which has been discussed; the effect upon the rate of delivery of blood to the tissue, the other.

Romberg states that the very rigidity of the walls interferes with the passage of the blood at the normal rate, while Allbutt opposes the idea that this can offer any great resistance and thus bring about a hypertension. Hasenfeld put forward the idea that arteriosclerosis of the splanchnic arteries might oppose a great resistance to the heart and it was actually shown by Longcope and McClintock that ligation of the splanchnic arteries produced a marked rise in blood pressure, while obstruction of other arteries had little or no effect. But Marchand showed that there is little relation between arteriosclerosis of the splanchnic arteries and cardiac hypertrophy. In general, it is difficult to correlate high blood pressure and cardiac hypertrophy with arteriosclerosis. In an analysis made by H. P. Smith of seventy-two cases at autopsy, chosen because the blood pressure was high, it was found impossible to show any parallelism between arteriosclerosis and blood pressure, while there is a very marked parallelism between the curves indicating the degree of nephritis, hypertension and cardiac hypertrophy. The lack of the auxiliary contractility of the arteries in driving on the blood into the capillaries, the lack of distensibility of the larger arteries, and the actual narrowing of certain points in many arteries by encroaching plaques, must offer some increased resistance to the heart but it seems insignificant in its effects upon the blood pressure and upon the heart itself when contrasted with the overwhelming effects of chronic nephritis. Heightening of the blood pressure seems to be a phenomenon actively produced by the circulatory apparatus and not the passive result of changes in the walls of the arteries.

With regard to the changes in the amount of blood delivered to the tissues by sclerotic arteries, we have no actual figures but there is much indirect evidence of a somewhat unsatisfactory kind.

The part played by sclerosis of the coronary vessels in the production of myocardial scars has been much discussed and all the evidence tends to show that (Fujinami, Strauch) in most cases at least the coronary branches leading to the scarred areas are not sclerotic. Nor is the matter made clear by the assumption that narrowing of the ostium of the coronary is responsible. In many cases the affected areas of the heart wall are in places scar-like, in other places soft, translucent and deep grayish-red. These seem to be early stages in the formation of a scar—the heart muscle fibers are lost but the granulation tissue which replaces them is extremely vascular. This in itself is inconsistent with the idea of scar formation on account of anemia, and probably is only one representative of the large group of cases in which causes other than restriction of the circulation (infections, toxins) have destroyed the myocardium.

Sclerosis of the vessels of the kidney in association with scarring of the kidney substance presents exactly the same problems. The statement is rather dogmatically made that sclerosis of the fine branches of the renal arteries is the cause of the degeneration and scarring of the kidneys in certain cases. Side by side with these are cases in which the kidneys are equally scarred and degenerated, in which there is little or no arteriosclerosis. It is as difficult to accept the idea that the arteriosclerosis causes the shrinkage of the kidney as the reverse. Some light seems to be thrown upon this by the indisputable instances in which the loss of function of an organ is followed by changes in the arteries which lead to their narrowing by a process of obliterative endarteritis and whether the destruction and loss of tissue be brought about by the climacterium or by age or by disease, there is the correlated effect upon the supplying arteries.

Etiology and pathogenesis of arteriosclerosis. Arteriosclerosis is one of those diseases difficult to explain because it develops so slowly through long years of life during which a great many possible causes have had an opportunity to affect the tissues. Our brilliant discoveries as to the etiology of disease come most readily when the transition from health to a pathological state is sudden and easily recognized by symptoms. Hence every conceivable idea has been expressed and tenaciously maintained with regard to this condition and many of them are so vague and ill-supported that it is wearisome to discuss them. None, however, is clearly demonstrated to have a definite bearing on the etiology of arteriosclerosis and we are quite as ignorant of its underlying cause as were our forefathers in the days of Morgagni.

If, however, we rehearse them once more it will be seen how little actual proof there is for the efficacy of these things in producing the disease. If we set them down in a table with the names of those who have expressed themselves for and against each theory, we find the columns about equally filled with names.

Mere old age can hardly be regarded as a separate factor in producing this condition since it is necessarily complicated by many other possibilities. Arteriosclerosis does not steadily increase with age as is shown by the analysis of many cases at autopsy by decades, although it may well be said that those dying in the later decades are in a sense selected persons who did not die of arteriosclerosis. It is found, however, in children and in a particularly highly developed form in young people. Romberg and Bäumler set aside old age as an essential factor.

Hereditary tendencies have been emphasized by Osler, Romberg, Fraenkel, Kisch and Hirsch but not regarded as important by v. Schrötter, Thoma and Edgren. Doctor Osler spoke of the quality of the arteries with which one came into the world.

Hard muscular work has been thought to favor the development of arteriosclerosis but the evidence is contradictory. While Weiss found it present in workers in foundries and factories, Edgren could not confirm this in farm laborers who are supposed to work hard. Of course, this too is not an isolated factor because hard bodily labor usually brings with it exposure to unhygienic housing conditions, abuse of alcohol and tobacco, syphilis and other infections and even in certain trades to toxic influences such as the fumes or dust from metallic and other poisons.

It is assumed to act through a heightening of blood pressure. Perhaps a rather pure example is that of Marchand who found in a young person who stood on one foot, the other leg being paralyzed, a marked sclerosis of the vessels of the overworked leg.

High blood pressure. This brings up the factor of heightened arterial pressure which is by one group regarded as the intermediary through which many of the other influences act. Sir Clifford Allbutt lays great stress upon the separation of those cases of arteriosclerosis which occur under conditions of heightened blood pressure (hyperpiesis) from those which occur in its absence and are found without hypertrophy of the heart usually in persons of avanced age (decrescent form). He does not believe that the arteriosclerosis itself is the cause of hypertension nor would he insist that hypertension is always followed by arteriosclerosis but without giving a convincing histological basis for their separation he maintains the distinction between these forms. It is of course common enough to find arteriosclerosis in cases of hypertension even without renal disease but as Lubarsch and Pal have shown and as we have all observed, there are cases of extreme hypertension without any arteriosclerosis. Where hypertension is local, as it can be in only a few places, as in the pulmonary artery in mitral stenosis, or when a communication between aorta and pulmonary artery is formed by the rupture of an aneurysm or by the ductus arteriosus in malformed hearts, we usually find sclerotic plaques in the vessel wall. Ljungdahl has studied this condition in many cases and finds that it is chiefly developed in those instances in which the artery is functionally overstrained. Mitral stenosis, congenital malformations and extreme pulmonary emphysema are the principal causes.

On the whole there is a widespread idea that heightened tension from whatever cause is a fundamental factor in the production of arteriosclerosis. It is assumed in the experimental studies carried out with adrenalin and with mechanical means of raising the pressure, it is used to account for the position of sclerotic plaques at the orifices of intercostal and other vessels, but in no case is it quite clear that the increased tension plays an essential part. On the contrary we find cases of nephritis with long standing high blood pressure with little or no arteriosclerosis, although hypertrophy of the muscular coat may exist. Klotz attempted to produce a work sclerosis by raising the blood pressure in the carotids and thoracic vessels by hanging a rabbit upside down each day for a long time. He succeeded in causing thereby an increased pressure in these vessels and extreme sclerotic changes. Others (Steinbiss, Miner Hill) failed to produce any changes by such means and Klotz himself did not always succeed. Harvey reached similar results to those of Klotz by compression of the aorta in a rabbit.

Apparently the element of hypertension does not enter into the conception of arteriosclerosis as a wear and tear disease of the vessels in which elastic tissue is replaced by connective tissue. Most of the names are associated with this idea from Rokitansky and Traube to Aschoff, Jores, Romberg and others. No one could deny the probability that such a replacement may occur, but the typical appearance of arteriosclerosis in the young with all its characteristic features makes it seem that this process has more individuality than mere wear and tear.

The same criticism may be offered for the expression, "increased functional demand" which is used by several to mean that under the stress of unusual activity the vessel wall suffers.

Numerous authors (Fraenkel, Huchard, Romberg, Weiss, Stengel) speak of mental activity or overactivity, mental diseases and various nervous disturbances as possibly productive of arteriosclerosis. That they may have an indirect bearing through disturbance of metabolism, or that they may be the expression of some intoxication which lies at the root of the arteriosclerosis, seems more plausible.

The association of certain diseases with arteriosclerosis is discussed but without producing very convincing evidence in its favor. Thus Huchard, Lancereaux, Fraenkel, Schrötter ascribe to gout a part in its production and others think that the same may be said of diabetes, chronic nephritis, obesity, etc., but in each case there are quite as many to deny the relation.

There remain perhaps the most important etiological factors—infections, intoxications and unbalanced diets.

The French school, including especially Huchard, have emphasized the importance of infectious processes in giving rise to arteriosclerosis. Typhoid fever, rheumatism, scarlet fever, diphtheria and influenza are especially frequently mentioned as being followed by the appearance of sclerotic lesions of the aorta. Thayer and his assistants, Brush and Fabyan, studied the cases of typhoid fever recalled for examination months or years after convalescence and found that they presented thickening of the peripheral arteries in a much higher percentage of cases than the controls; and at autopsy he found in deaths from typhoid fever fresh sclerotic plaques in the aorta and coronary arteries. Earlier workers, such as H. Martin, Therese, Simnitský, had recorded observations pointing to the occurrence of lesions in the arteries after infections which might possibly lead to arteriosclerosis. Wiesel studied a large number of cases in young persons who had died of acute infections and found minute necroses in the media which sometimes showed as sunken spots in the lining of the vessel. Frothingham made similar observations in diphtheria, typhoid fever, glanders and other diseases. These papers have been reviewed by Ophüls in an excellent monograph on the relation of arteriosclerosis to infectious disease in which he shows by the careful study of five hundred cases that arteriosclerosis develops in connection with injury to the arteries resulting from various infections of which chronic septic infections of the rheumatic type play the most important rôle.

Comparison of tables made up of cases in which there was a history of one or more infections, with those made up from persons who had suffered little or no infection, shows an extraordinary preponderance of arteriosclerosis in the former.

Ophüls feels that it is misleading and confusing to consider the renal lesions so frequently associated with arteriosclerosis as a result of changes in the arteries or vice versa. They are more probably produced by the same causative agent. Nor does he regard hypertension and arteriosclerosis, which sometimes occur together, as interdependent.

Naturally attempts have been made to reproduce the arteriosclerotic lesions supposed to follow infections by experimental inoculations, either with living or dead bacteria or with their extracts or toxins, and many investigators have been able to produce lesions of the arteries rather closely resembling those of arteriosclerosis. Gilbert and Lion did this with typhoid bacilli and with an organism from a case of endocarditis. Boinet and Romary, Therese, Crocq, Manouelian and Saltykow continued these experiments. Most of these worked with rabbits and so did Klotz and Bailey, who produced distinct arterial lesions with calcification by the injection of diphtheria toxin. These animals are so prone to spontaneous arterial lesions that it seems most unfortunate that they should always be chosen by experimenters although it may be supposed that in any considerable series of positive results criticism of this sort is largely disarmed. But for this reason the injection of killed staphylococci or filtrates by Manouelian into monkeys with the production of medial lesions seems to assume a somewhat greater importance.

Nevertheless, in spite of all these experiments and these statistical studies of the co-existence or later occurrence of arteriosclerotic lesions in persons affected with infectious diseases, there is brought no satisfactory proof of the thesis that infection is the direct cause of arteriosclerosis. It seems the most plausible idea so far mentioned but it will require far more study before we see the relation clearly.

With regard to the part played by toxic substances of various sorts the most general statements are made by Huchard, Romberg, Craig, Senator and others who variously supposed the occurrence of poisonous materials capable of causing a vasomotor spasm with high blood pressure and ultimately arteriosclerosis. Craig's idea was that this was a form of auto-intoxication from the intestine. More specific arguments have been put forward by Billings, Huchard and Lunz concerning the influence of lead poisoning but Jores and Kolisko have been unable to substantiate its importance in producing arteriosclerosis. The association in this and in the following instances is so vague that it seems hardly worth while to analyze the statements more exactly. To tobacco has been ascribed the same effect by a number of well-known authors, Schrötter, Huchard, Romberg, Schott, Külbs, and especially by Adler and Hensel. Doctor Adler was able to produce arterial lesions in dogs by the injection of relatively enormous doses of nicotine but this can scarcely be taken as a proof of the inimical effect of smoking in man. Edgren and v. Basch refuse to accept the supposed etiological importance of tobacco in this disease. It is not impossible that nicotine may have some direct or favoring influence in the production of arteriosclerosis but the evidence is less than circumstantial. The same may be said of tea and coffee—there are voices for and against assigning them any importance. Alcohol has always been prominently mentioned as a causative factor usually with the further explanation that it acts by elevating the blood pressure or by causing sudden variations in the pressure. Edgren, Hirsch, Gerhardt, Schrötter, Chiari, Klemperer believed in its importance but Romberg, and especially Cabot, have been unable to show that among patients with arteriosclerosis there is any extraordinary percentage of alcoholism nor vice versa.

Since the idea that hypertension could bring about arteriosclerosis has for a long time been very familiar, it was natural that experimental attempts to test this with adrenalin, whose power of producing vasoconstriction and high blood pressure was well known, should have been attempted. Jores' experiments in this direction failed but Josue was able to cause in rabbits the formation in the aorta of localized areas of medial necrosis with dilatation and calcification. Numerous investigators followed with the same results (Erb, Pearce and Stanton, Braun, Klotz, Loeb, Githens and Fleisher, and many others). All found that areas of necrosis appear in the media which may involve its whole thickness or only a part. The artery may be dilated at this point—the necrotic tissue becomes calcified and surrounded by a granulation tissue often with giant cells, as might be the case with a foreign body. A compensatory new formation of intimal tissue which ultimately levels the intimal surface may occur. The descriptions are practically always the same and while this condition is not regarded as identical with the arteriosclerotic lesions of the human aorta, they do resemble closely the Mönckeberg type of medial calcification found in the peripheral arteries in human beings.

Such disease of the aorta occurs so often spontaneously in the rabbit's aorta that Hill thinks it necessary to have a very large series before the lesion can be accepted as the result of the adrenalin injection. But by this time the unanimous reports of many workers constitute a large series and it seems that it may be accepted that this is the effect of the injection. Whether it is the effect of the blood pressure raising influence of the adrenalin has been studied in various ways, chiefly by adding amyl nitrite or other substance which will tend to neutralize this violent pressor effect. Even then the same arterial lesions are produced (Klotz), so that it appears that some toxic action of the adrenalin and not merely a mechanical effect of hypertension is at work.

A minute analysis of all the work upon this subject up to 1908 has been given by Saltykow and the question as to its bearing upon human arteriosclerosis seems to be settled.

Various other substances have been injected in the effort to produce experimentally arteriosclerosis, usually with the result that changes of some sort are produced but never resembling very closely arteriosclerosis. Such substances as uric acid, sodium urate, barium, ergotin, hydrastin, mercury and extracts of decomposed meat have given no consistent results.

Far more interesting are the experiments begun by Ignatowski and carried out in detail by a number of other investigators, Anitschkow and Chalatow, Starokadomsky and Ssobolew, Stuckey, Wacker and Hueck, but especially Anitschkoff, upon the effect of modifications of the diet. These modifications consist chiefly in the introduction of animal food into the diet of the vegetarian rabbit and especially in the increase in the proportion of lipoid substances. Most of these authors have ultimately investigated the effect of the feeding of cholesterin and cholesterin esters although a few have been interested in the effects of high protein diets. Anitschkow in his later paper begins by showing that the experiments of Saltykow in which staphylococci were injected over long periods were complicated by milk feeding which alone will produce the changes in the aorta accompanied by deposits of fat, while the bacteria alone without simultaneous milk feeding will not do so (Herxheimer, Reddingius and Starokadomsky).

In his own experiments in which he fed cholesterin dissolved in sunflower seed oil he thinks he has produced a condition which in respect to the deposition of fat and the hyperplasia of the intima closely resembles human arteriosclerosis in sharp contrast with the changes of the adrenalin type which bear no close resemblance to the human disease.

In these experiments, first with the feeding of cholesterin alone, then the same feeding combined with mechanical narrowing of the aorta by a ligature, then with suspension of the rabbits in inverted position (Klotz) with and without cholesterin feeding, he finds as the clear-cut result that the mechanical interference which would lead to heightened blood pressure alone fails to produce the characteristic changes in the vessel wall, while these appear when cholesterin feeding is combined with them and indeed after the feeding of smaller amounts of cholesterin than are necessary to produce atherosclerotic changes in the walls when given in normal resting rabbits. There is then something in the heightening of the blood pressure (after all no great increase in blood pressure is produced) which favors the deposition of the lipoid and the development of the hyperplastic changes. Similar results are recorded for a combination of cholesterin with adrenalin injections but this seems a confusing experiment because different end results are combined.

Anitschkow's view is, therefore, that the inception of arteriosclerosis is probably to be ascribed to the storing of lipoid substances in the intima but that this alone will not occur except as a process of summation with various other predisposing causes of which he has tested one or two examples. He expresses then a combination theory of the origin of arteriosclerosis in man.

SUMMARY

On the whole the anatomical changes in arteriosclerosis are now becoming fairly clear and there is almost complete unanimity of opinion as to what should be included under this name and what excluded. Syphilitic and other infectious forms are excluded, obliterating forms of endarteritis are usually easily classed apart with well understood cause and quite separate anatomical characters and there remains only the "ordinary" arteriosclerosis with the somewhat more obscure division formed by the medial necrosis in peripheral vessels. This form of Mönckeberg seems very distinct from the other, more nearly related to the forms of obliterating endarteritis and often combined with it. It has, therefore, been little considered here.

In the true arteriosclerosis there seems to emerge from the long continued discussion the general conception of the paramount importance of the intimal changes although on grounds of probability, weakening of the media has always been sought. The presence of lipoid substances in the intima followed by a great hyperplasia of the connective tissue and to a less extent of the elastic tissue is agreed upon. Every conceivable explanation has been offered, based usually on a preformed idea as to what ought to occur. Every form of experiment has been performed in the attempt to reproduce the disease. These resolve themselves into three main groups:

1. Efforts toward affecting the vessel wall by mechanical means through elevation of the blood pressure or otherwise.
2. Efforts toward producing lesions in the vessel wall by various poisons.

3. Experiments to show the effects of perverted or one-sided diets.

Of these practically all that fall in the first two groups have failed to produce anything like human arteriosclerosis, while those of the third group have been more successful, especially when combined with mechanical disturbances. Of all the mechanical influences it seems that heightened blood pressure is the only one of any importance but that acting alone it produces little or no change. At most it seems to predispose to the imbibition of excessive lipoids in the intimal layer; whether these lipoids themselves are the cause of the tissue hyperplasia remains to be determined.

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FAT TRANSPORT IN THE ANIMAL BODY

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The essential constituents of the proteins and carbohydrates—the amino acids and the monosaccharides—are soluble in water, while those of the fats—the higher fatty acids—are not. The amino acids and monosaccharides are, according to our most recent knowledge, carried as such in the blood, while the split products of the fats are built up again into fat for transport. The hydrolysis products of the proteins and carbohydrates pass directly into the blood stream, while it has never been shown that any of the fat goes that way, although it is probable that a small amount does. For these reasons and probably also for others as yet not understood, arrangements are provided in the organism for transporting the fats which are in most respects quite different from those provided for the other foodstuffs. It is proposed in the present discussion to review the available information on this subject.

TRANSPORT ACROSS THE INTESTINAL WALL. In common with all the other food substances the fats are hydrolyzed in the gastro-intestinal canal, the products of the hydrolysis being fatty acids and glycerol. The extent of hydrolysis has been a matter of dispute but ordinarily conditions are such that it is probably nearly, if not quite complete. There is abundance of lipase, mainly from the pancreas but also from the intestinal glands, to hydrolyze many times the amount of fat ordinarily found in the diet. Due to the mechanism for emptying the stomach, the fat is supplied to the intestine in small amounts. There is always some free fatty acid in it when it reaches the intestine since all natural fat contains fatty acid, and the amount is increased by cooking and probably also by the gastric lipase. An abundant supply of alkali is furnished by the various secretions which empty into the intestine—bile, pancreatic and intestinal secretions—and the alkali uniting with the fatty acids forms soaps, by the aid of which the unsaponified fat is emulsified so as to be the more readily hydrolyzable. Added to these factors is the removal of the products of hydrolysis by absorption, which, since hydrolysis is a reversible reaction, promotes its completeness. In the face of so complete a mechanism for hydrolysis the conclusion is almost inevitable that, whatever be their later fate, the fats are ordinarily completely saponified in the intestine. On the other hand the structure of the absorbing surface of the intestine, the presence there in enormous number of leucocytes which are generally loaded with fat and which are known to pass through the intestinal epithelium, the fact that non-motile bacteria pass across the intestine when fed along with fat, the histological picture of the epithelial cells during fat absorption, which although perhaps better explained by the absorption of fat as split products, does not preclude the absorption of unsplit fat in an ultramicroscopic state of division, together with the fact that only fat is found in the lacteals and thoracic duct, make it unsafe to assume that fat is absorbed wholly in the hydrolyzed form and renders desirable also the careful consideration of the possibility of the passage of unaltered fat across the intestinal wall.

The histological study of fat absorption. The most important investigations on this subject are those of Schaefer and Heidenhain who, however, arrive at opposite conclusions as to the way in which the fat passes across the intestinal walls. Recent work by Clark and Clark and by Evans, while not directly concerned with fat absorption from the intestine, is still of great importance in its consideration.

Schaefer (1), in discussing the phenomena occurring in the intestinal epithelium during fat absorption, first noted that there is no definite or at least no rigid cell membrane surrounding the columnar epithelial cells of the villi; that soft bodied cells such as leucocytes occurring between them are able to indent and to work their way through between them. During fat absorption the epithelial cells become filled with fat globules of various sizes, generally largest in the part between the nucleus and the thickened border, and often quite small near the attached end of the cell. Sometimes the greater part of the fat is accumulated in the inner, sometimes in the outer part of the cell, these conditions probably representing the different stages in its absorption. The appearance shown in different preparations is such as to indicate a primary accumulation of fat in the outer or free half of the cell, and its gradual passage down into the inner or attached half, accompanied by the breaking down of the larger particles into smaller ones preliminary to passage out of the cell. The striated outer border (near the lumen) has been the object of a good deal of interest in connection with the passage of fat into the epithelial cells. A few investigators from Wiedersheim (2) onward claim to have observed what appear to be amoeboid protrusions from the cell border into the intestine and have ascribed to these processes the function of engulfing fat particles from the intestine and transferring them to the interior of the cell. This idea of the method of entry of fat particles into the cells is given sufficient credit by histologists to be included in at least one modern text book (3). However, most investigators have been unable to find even a trace of such outgrowths and the possibility, if present, of their having any function in fat absorption is remote, for the reasons that if they could engulf fat particles they could also take in molecules of starch or protein, which are much smaller, but which are known not to pass the normal intestine; also that the appearance of the fat particles in the cells represents a gradual growth rather than a sudden transference, and that fat globules have never been observed in the substance of the striated border, although such an appearance would be frequent if the globules did pass through this border. Schaefer has found that leucocytes accumulate in the mucous membrane in great numbers during fat absorption and he believes them to be of great importance, if not the main factor in the transference of fat from the epithelial cells to the lymph system. His claim assumes the greater weight in the light of recent work by Clark and Clark (see below). The leucocytes are generally very abundant at the base of the epithelial cells, between them and the basement membrane, but are less abundant between the cells, and occur only occasionally in the intestinal lumen. They are present in large numbers in the interior of the villi and occasionally in the lacteals, especially near the end. That they pass from the villi into the lacteals is shown by the fact that they may be seen partially in- and partially outside the lacteal. During fat absorption they are always filled with fat globules, even though the epithelial cells may not be. Schaefer ascribes the main transport of fat from the epithelium to the lymphatics to these cells, and believes that the epithelial cells becoming visibly filled with fat only when the leucocytes fall behind with their work of transport, acting thus as workshops in which the absorbed fat constituents—glycerol and fatty acid or soap—are recombined as fat and stored against the time of removal by the leucocytes. In Schaefer's opinion the leucocytes, after carrying their load of fat into the lacteals, break down, since although they are found in considerable numbers in the lacteals near their beginning, they cannot be found in the thoracic duct. Schaefer's findings and deductions thus provide a complete hypothesis as to the passage of fat from the intestine into the circulation. Its acceptance is based on the ability of the leucocytes to do the work, which may be questioned, although they undoubtedly are an important factor. From his hypothesis the inference follows that the manner of passage of fat through the two surfaces of the epithelial cell is different—absorption into the cell taking place as fatty acid and glycerol, while passage out into the leucocytes is as finely particulate fat.[1] The transference of fat in a very fine state of division—as an invisible colloidal suspension—has never been considered and is a fruitful possibility, since living cells appear to have the ability not only of building up large globules of fat but also of reducing these again to invisibility.

Heidenhain (5) differs from Schaefer in some important particulars as to his explanation of fat absorption. Commenting on the claims of Zawarykin (6) who believed that the leucocytes alone were concerned with the transfer of fat from the intestine to the lacteals and that the epithelial cells took no part, he admits that the leucocytes undoubtedly can take up fat from the intestine, but believes their part to be secondary for the following reasons: *a*, In newborn puppies when fat absorption is in full progress leucocytes are seldom found in the epithelium, while in fasting they are often present in large numbers. *b*, Leucocytes containing granules stained black with osmic acid are often found in the Lieberkuhn's glands and their presence is difficult to explain since there is no apparent reason why they should transport fat there and the glands themselves do not absorb fat. *c*, Granules staining black with osmic acid may be found in fasting animals near Lieberkuhn's glands and less numerously in the gland itself, and evidence is brought to show that probably some substance other than fat is responsible for the color with osmic acid.

The presence of fat between the epithelial cells observed by some workers (7) Heidenhain is inclined to regard as due to muscular contractions of the villi during fixing, since it cannot be observed in the epithelial cells of animals whose villi have no muscles (frog).

The absorption of unchanged fat through the agency of outgrowths of the epithelial cells of the nature of cilia as claimed by Thanhofer (8) he believes to be very doubtful because of the inability of many histologists (himself included) to demonstrate the presence of anything of the nature of cilia.

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[1] Regarding the necessity for hydrolysis of the fat before absorption, one reason is probably that of protecting the organism from unsaponifiable fatty materials (4) but once having passed the test of saponification it is very possible that fat may then not have to submit to hydrolysis again but may be passed along in particulate form.

For the transfer of fat from the epithelial cells it is his belief that contraction of the protoplasm is responsible as is the case for the transfer of water.

Inside the villi the fat, as relatively coarse globules, moves in the pericellular fluid contents (with the exception of the *small* amount taken up by the leucocytes) and does not assume the dustlike fineness of its final form until it reaches the chyle vessels.

Clark and Clark (9) have recently presented some experiments which are of great interest in connection with the rôle of the leucocytes in fat transport in the intestine and elsewhere, and are in striking support of the claims of Schaefer as regards this function. Drops of fat or fatty acid 30 to 70μ in diameter (olive oil, cream, yolk of egg, oleic acid) were injected into the tissue of the tails of tadpoles and the resulting reactions noted. In the case of olive oil, soon after the injection, leucocytes were observed to pass through the walls of the nearby blood vessels and to wander toward the oil droplet. On reaching it they flattened out, formed a ring about it and in a few minutes became pigmented, the pigment being apparently minute particles of oil. Lymph vessels grew out to the oil in from a few hours to two or three days, depending on the distance, and remained in contact with the oil and the leucocytes for several days. No pigmented leucocytes were seen to enter the lymphatic, but the oil droplets in the leucocyte in contact with the lymph vessel became gradually smaller until the cell became clear. Fine, free fat droplets were engulfed by leucocytes, were then reduced in size and replaced by minute pigmented droplets.

In the case of oleic acid, within a minute or two after injection, the clear globule became opaque and granular—brown by transmitted light The leucocytes responded more quickly and in larger numbers than with olive oil (irritation by the fatty acids?) forming a ring several layers deep and soon becoming deeply pigmented. The lymph vessels responded as with the olive oil and a study of the leucocytes showed that they were continually moving away from the fatty acid, wandering up to a nearby lymphatic and in fifteen minutes to one half-hour moving away, having lost their brown pigment. None were observed to enter the lymph vessel Absorption of oleic acid or sodium oleate was more rapid than olive oil.

When cream or yolk of egg (finely divided, emulsified fat) was injected, the same reactions were observed—the leucocytes acting as carriers but working in this case more rapidly. When injection was near a lymph capillary, loaded leucocytes came into contact with the lymphatic within three hours after the injection. If, however, the leucocytes had far to go they lost their pigment (and therefore their load of fat?) before reaching the lymph vessel, which however continued to grow in their direction and that of the fat mass, indicating perhaps a diffusion of soluble and therefore invisible products (hydrolysis products?) from the leucocyte. The absorption of cream or yolk of egg was so rapid that there was often no time for the lymphatic to grow out to the injected material. Leucocytes and lymphatics were the only structures reacting to the injected fat, the blood capillaries, if anything, growing away from it. These reactions were limited to fat and fatty acid. No reaction could be obtained with fat-like substances such as mineral oil.

The work of Clark and Clark indicates that the leucocytes can take up unsplit fat and transport it fairly rapidly, and their presence in the intestine in such large numbers during fat absorption and the fact that they are always loaded with fat even when the epithelial cells contain very little, indicates that they may have a very considerable part in certain stages of the absorption, i.e., transport to the lacteals. But numerous as they are in the lacteals and around the epithelial cells they rarely get out into the lumen of the intestine, so that the first stages at least of the absorption of fat must be referred to the epithelial cells themselves. The possibility that the epithelial cells act phagocytically, engulfing the unchanged fat particles, seems doubtful from the fact that fat particles have never been observed in the cuticular membrane, and that most of the considerable mass of evidence available points to the absorption of fat in the form of its hydrolytic products—glycerol and fatty acids (or soaps).

Form in which fat leaves the intestine. The experimental data regarding the form in which fat passes out of the intestine may be considered under four headings; first, that bearing on the degree of hydrolysis of fat in the intestine; second, that regarding the absorbability of the split products; third, the behavior of the intestine toward substances of a fatty nature which are not fat; and fourth, the evidence regarding the absorption of unchanged fat.

1. The extent of fat hydrolysis in the intestine. No matter how much fat is passing along the intestine or how little is being absorbed, the fatty material found in the lower intestine and the feces consists almost entirely of fatty acids (or soaps) (10), which indicates clearly that ample facilities are provided for hydrolysis. When absorption is taking place with consequent removal of the products, hydrolysis must be even more complete, since it is a reversible process and follows the law of mass action. In healthy animals enough pancreatic lipase is provided to split many times the amount of fat ordinarily present in the food. Added to this main source of lipase is that present in the gastric secretion (11) which, although not ordinarily important because of its sensitiveness to acid, may bring about considerable hydrolysis when the gastric acidity is low and especially when fat is present in an emulsified form (milk, yolk of egg, etc.). In addition to the gastric lipase some is supplied in the intestinal secretions (12), which is probably responsible for the splitting of fat which is found to take place in the absence of the pancreatic secretion.

Owing to the fact that the lipases are soluble in watery mixtures but not in fat, their hydrolytic action can be exerted only at the fat-water interfaces, and unless the fat surface thus exposed is great, splitting will be slow. Hence the importance of emulsification, which by reducing the size of the fat globules increases their surface. Abundant provision is made for emulsification in the intestine. The most important emulsifying agent is undoubtedly soap, the fatty acid for the formation of which is present to a small extent in all natural fats and the amount is increased by cooking and by the gastric lipase. Alkali is supplied by the secretions entering the intestine—pancreatic juice, bile and the various intestinal juices which are all more or less alkaline. The fat containing fatty acid ordinarily enters the intestine in small portions, is met by the alkaline secretions which unite with the fatty acid to form soap and by the motion of the intestine the unsplit fat is well emulsified. Additional emulsifying agents or stabilizers—proteins, lecithin, etc., are furnished by the secretions, and even though, as has been noted several times, the reaction of the intestine is slightly acid throughout its length (13), the acidity is due mainly to carbonic acid, which has little effect on a soap emulsion in the presence of bile and pancreatic secretion (14). The fact that it is not always possible to find an emulsion in the intestine during fat absorption (15) is not a serious objection since such a finding would be made if hydrolysis were rapid in relation to the amount of fat present—all the fat present being in the form of soap.

2. The absorbability of the split products of fat—fatty acids or soaps and glycerol. That the split products of fat were absorbable was demonstrated very early in the study of the behavior of fat in the intestine.

Radziejewski (16) showed that alkali soaps were absorbed; Perewoznikoff (17) showed that a mixture of alkali soap and glycerol was absorbed and synthesized into fat, the lacteals having the usual appearance after a fat meal and the epithelial cells containing fat globules. All the more recent evidence confirms the earlier findings (18) and the fact seems proven that the intestine can absorb fatty acids or soaps and glycerol and synthesize fat from them.

3. The behavior of the intestine toward substances of a fatty nature other than ordinary fat. A number of workers have experimented with substances of this type in the hope that thereby information regarding the manner of absorption of fat might be obtained, and the results have been quite illuminating. *a.* Substances which are hydrolyzable in the intestine—ethyl esters of the fatty acids (19), amyl esters of the fatty acids (18), optically active mannite esters of the fatty acids (20). These are all completely hydrolyzed in the intestine and the fatty acid component appears in the chyle as the triglyceride showing that a synthesis with glycerol has taken place. *b.* Non-hydrolyzable substances which by one means or another can be emulsified. Two substances have been used for this purpose—esters of the fatty acids with cholesterol or related substances which readily yield a fine emulsion with water, and the paraffin hydrocarbons which are soluble in fat and which can be emulsified with it. Absorption of these substances has been tested in two ways—by determinations of unabsorbed residue in the feces (21) and by determining the absorbed substance in the chyle (22). In neither case was there any evidence of absorption. A similar rejection of paraffin hydrocarbons was observed by Clark and Clark in their study of the absorption of fat and fat-like substances injected into the tails of tadpoles.

4. Absorption of unhydrolyzed fat. There is no good evidence that fats can be absorbed unchanged in any considerable amount. The belief that such was the case was based largely on the observation that the appearance of the fat in the intestine and the lacteals was the same—a milky emulsion. The objection, that the particles of suspended fat in the lacteals were much smaller than those in the intestine has never been explained away but a reasonable answer would be that only the finer particles of the fat of the intestine were absorbed, and that one of the purposes of intestinal action was to reduce the fat particles to absorbable size, the processes of hydrolysis and emulsification being directed to that end. Hydrolysis of fat by the lipases in the intestine was never denied by the supporters of the theory that fat was absorbed as such in finely divided form but their claim was that only enough hydrolysis took place to produce soap for emulsification. The repeated statements of the histologist that no fat particles can be observed in the cuticular membrane which is the first line of action of the epithelial cells have never been answered and could be explained only by the assumption that the fat particles were so fine as to be ultramicroscopic which, so far as is known, has never been made. There appears to be nothing inherently impossible in the reduction of fat particles to ultramicroscopic size since fat is known to occur in such a (colloidal) condition in nature as stored material in egg yolk and in many cells, both plant and animal, and as noted above, the reduction in size of fat particles to invisibility has been observed in leucocytes and in intestinal epithelial cells. But the ability to reduce fat to particles of ultramicroscopic size appears to be confined to the living interior of cells and could probably not take place in the intestine. Even if it were possible to reduce fat to the colloidal condition in the intestine a special mechanism must still be assumed for its absorption since other substances in colloidal suspension such as proteins and starches and even relatively simple substances in true solution such as the various disaccharides are not allowed to pass into the blood unchanged. Other evidence of absorption of unchanged fat such as the absorption of colors which are soluble in fat but not in water, and the deposition in the fat stores under certain conditions of large amounts of fat which is chemically the same as the fat of the food can be equally well explained by the hydrolysis-synthesis theory, since the fat dyes used were shown to be soluble in fatty acids (23), and since the fat found in the lacteals and hence in the blood and fat stores must be built up largely from the available hydrolysis products of the fat in the intestine.

To sum up: Abundant facilities are provided for the hydrolysis of those esters of the fatty acids which hydrolyze with the same or greater ease than the fats. The split products are readily absorbed and converted into fat in the passage through the intestinal wall. Substances which cannot be hydrolyzed and so rendered water-soluble are not absorbed no matter in what form they may be presented. The prevailing belief that fats are completely hydrolyzed in the intestine and absorbed as the hydrolysis products thus has most of the evidence in its favor, although the possibility of the absorption of some unchanged fat cannot be absolutely excluded. The reason for the hydrolysis, which appears to be universal for all food substances, is not far to seek. On the one hand there is the necessity for the exclusion of substances which as presented would be useless and harmful to the organism, such as unchanged proteins, complex carbohydrates or fat-like substances other than fat; and on the other, since no food substance is presented in immediately usable form, more or less complete hydrolysis must take place in any case, if not in the intestine then in the tissues. The two purposes are combined in the intestine, which by means of the same process rejects harmful or useless substances and reduces the useful material to fragments which can be used at once by the tissue cells which require them. There is therefore no need to assume a special mechanism for the transfer of fat from the intestinal lumen into the epithelial cells—this takes place according to the usual rule by hydrolysis, which is as usual complete to the limits of hydrolysis for the individual substance, followed by absorption of the split products in water solution. (Attention should be called to the fact, which is perhaps not ordinarily sufficiently appreciated, that hydrolysis in the intestine is carried to the utmost limit; any further change such as division of the dextrose molecule, deamination of the amino acids or breaking of the fatty acid chain would involve other changes than simple hydrolysis.)

Passage of fat out of the epithelial cells. The fat fragments after reaching the interior of the epithelial cell appear to be built up into fat again and retained in the cells for some time—at least this is the picture when much fat is being absorbed. When little absorption is taking place no such accumulation can be observed, but the leucocytes, according to Schaefer's observations, are always full of fat whether much or little is being absorbed, and his belief is that the function of the epithelial cells is to receive the hydrolysis products, synthesize them and store the fat until the leucocytes can transport it to the lacteals. Both Schaefer and Heidenhain agree that the fat from the epithelial cells is transferred to the lacteals without alteration other than changes in size of particles, Heidenhain believing that the particles are expelled into the body of the villus by contractions of the epithelial cell protoplasm, while Schaefer thinks that they are transferred to the leucocytes by contact and by them to the lacteals.

In contrast to these opinions based on histological observations, Loevenhart (24), on the basis of chemical evidence, believes that the passage out of the cell, like the passage into it, is accomplished by a hydrolysis and synthesis, and also that wherever in its subsequent history the fat has to pass a cell wall the same hydrolysis and synthesis take place. His assumption requires the presence at all these points of a sufficient supply of lipase to bring about these changes in the time ordinarily consumed in transferring the fat from the intestine to the tissues and he brings evidence to show that lipase is present in all tissues, and especially in those which are ordinarily most concerned in fat metabolism—the liver, active mammary gland, blood, lymph and intestinal mucosa. He notes particularly its presence in those places where fat synthesis is known to take place—the active mammary gland and the subcutaneous fatty tissue. The test employed by him to show the presence of lipase is the ability of a water extract of the tissue to hydrolyze ethyl butyrate. Aside from the fact that the splitting obtained by him is rarely very extensive, being ordinarily less than 5 per cent in forty hours and therefore possibly unimportant as a factor in fat metabolism, his experiments have aroused critical comment in several quarters. Regarding the use of ethyl butyrate as a test of the presence of lipase, it was pointed out by Arthus (25) and later by Jansen (26) that a test carried out on the esters of the lower fatty acids such as monobutyrin (and therefore also ethyl butyrate) cannot give a true measure of lipase action since these esters are much more readily hydrolyzed than the triglycerides of the higher fatty acids and regarding which Loevenhart himself gives a reference to Euler (27) to the effect that the hydrolysis of an ester is greater the stronger the constituent acid; therefore the higher fatty acids being relatively much weaker than butyric acid their esters would be much more slowly hydrolyzed than those of butyric acid.

Bradley (28), using the same technique as Loevenhart, was unable to corroborate his findings in some important particulars. He found no broad correlation between fat and lipase content of tissues. Some of the most active fat-producing tissues (mammary gland) are relatively poorer in lipase than others which never normally contain or produce more than a small percentage of fat (lung, kidney, muscle). He concludes that quantitative comparison of fat and lipase in animal tissue gives no positive evidence in support of the theory of enzyme synthesis.

Thiele (29) found that blood and chyle contain a ferment which can hydrolyze phosphatides but not fat, and also (30) that tissue extracts (excluding pancreatic extract) similarly cannot hydrolyze fat but can hydrolyze the phosphatides.

Porter (31) found ferments capable of splitting ordinary fat (olein, stearin) present in most tissues but generally in the merest traces, except in the case of the pancreas, the amount of splitting taking place being so small as to be of no practical significance in a consideration of fat metabolism. Ferments capable of splitting lecithin were present in larger amounts.

In earlier work Kastle and Loevenhart (32) found that pancreatic extract was less active on ethyl butyrate than liver extract but the reverse was the case when fats were used as zymolytes.

It appears desirable therefore to differentiate between enzymes which hydrolyze the fats readily—the true lipases—and those which work slowly on the fats but readily on the esters of the lower fatty acids and on lecithin and which may be called esterases. Lipases appear to be present in significant amounts only in the intestinal secretions of the pancreas and other intestinal glands, while the esterases are of quite general distribution. In view of the importance of lecithin as an intermediate stage in fat metabolism the esterases are therefore to be regarded as potentially of considerable significance. Since fat is synthesized in the intestinal epithelium, the presence there of a true lipase must, however, be admitted and the question of the passage of fat out of these cells into the villi or into the leucocytes by the hydrolysis-synthesis method must be left open.

The bile in fat absorption. Along with the abundant lipase supplied to the intestine there is secreted a similarly large volume of bile, the importance of which in fat absorption has been known for a long time. Although it furnishes no lipase, its exclusion from the intestine results in as much or greater loss of fat than the loss of the pancreatic secretion. Its main function appears to be in increasing the solubility of the fatty acids and soaps in the intestine and so aiding their transport through the absorbing cells (33). It also increases the activity of the lipases (34), increases intestinal peristalsis and provides materials which aid in providing optimum conditions for fat digestion. These later are alkali (carbonate and bicarbonate) to be used in the formation of soaps and in preserving the reaction of the intestinal fluids, and lecithin, mucin, etc., which stabilize the fat emulsions.

Considering all the evidence together, there is every reason to believe that the fat is completely hydrolyzed into glycerol and fatty acids before it passes from the intestine. The split products are taken up by the epithelial cells and converted by them into fat which, depending on the rate of inflow from the intestine, is either passed on at once to the lacteals or stored temporarily. As to what happens to the fat from this time on to its appearance in the thoracic duct very little is known with certainty beyond the fact that before passing out of the epithelial cells the fat particles are reduced to invisibility. Whether this means re-hydrolysis or whether the division is a physical one is not clear, but since fat is synthesized in these cells there must be lipase present and re-hydrolysis is at least a possibility. Heidenhain's claim that the fat particles are expelled from the epithelial cells by contraction of the protoplasm is difficult to accept since the cells are fixed at the basal end and there is no more reason to assume an amoeboid character at this end than at the other. The leucocytes, in view of the work of Schaefer and later of Clark and Clark, undoubtedly do carry fat to the lacteals, but whether their function is of importance in intestinal absorption remains to be shown, the whole question depending on whether there are enough of them to do the work. As shown by Clark and Clark, the leucocytes have the power, like the epithelial cells, of building up large fat globules in their bodies and also of reducing them again to invisible size, but whether this involves a double hydrolysis and synthesis also remains to be proven, since lipase in significant amounts has not been demonstrated. Owing to the absence of lipase in amount sufficient to bring about these numerous hydrolyses and syntheses in the various tissues it appears better for the present to assume that the primary hydrolysis in the intestine and synthesis in the epithelial cells is the only one the fats undergo and that all further transport across cell walls is either as particulate matter in a fine state of division or by transformation into water-soluble substances of the nature of lecithin, for the hydrolysis (and therefore synthesis) of which enzymes are available. As will be seen later, both methods are probably employed.

Passage of fat into the circulation. Fat absorption is generally discussed only in connection with the passage of fat into the lacteals and the thoracic duct and so to the circulation indirectly since this seems to be the main and, up to the present, the only demonstrable path of absorption. What are the indications that fat may pass into the circulation in other and possibly more direct ways? It has never been possible to recover all the absorbed fat from the chyle of the thoracic duct, in fact about 60 per cent appears to be the maximum recoverable (35),—which would indicate either that some of the absorbed fat had been stored somewhere along the lymph tract or that some had been absorbed directly into the blood stream. Evidence regarding absorption directly into the blood stream from the intestine is contradictory. Bornstein, under Heidenhain's direction (36), compared the fat content of the carotid and portal blood at the height of fat absorption and found the fat content of the carotid artery to be slightly higher both in whole blood and in the dry residue than in the portal vein. D'Errico (37) found that during normal fat absorption the total solids and the fat content of the portal vein were always higher than those of the jugular.

After ligature of the thoracic duct the fat content of the dry residue of the portal vein was still slightly higher than that of the jugular, but nothing like as high as would be expected. D'Errico's experiments may be criticised from the fact that samples were not taken simultaneously but from one-half to one hour apart, and that absorption of all kinds from the intestine was interfered with by the operation since the total solids in the portal vein diminishes considerably. These experiments have been repeated recently by Zucker (38), with negative results. He ascribes d'Errico's positive results to faulty technique and concludes that no marked participation of the blood vessels in fat absorption can be assumed. It seems therefore that although the possibility of absorption of fat in small amounts by the blood vessels cannot be denied, there is no evidence yet available to prove it. It should be noted however that none of the methods used for the determination of fat can be relied on to give results accurate to within much less than 5 per cent of the true value, and in view of the extensive circulation through the portal system a difference of 2 or 3 per cent would account for a considerable absorption. It is perhaps significant in this connection that Clark and Clark found no attraction between blood capillaries and fat or leucocytes such as is exhibited between them and lymph capillaries. Excluding direct absorption into the blood the 30 to 40 per cent of absorbed fat which cannot be collected from the thoracic duct may be accounted for either by storage in some place along the lymph path or by entry into the lymph or blood systems by other channels than the thoracic duct. No such connection has ever been demonstrated and if present it is probably of little importance since when the thoracic duct is tied off little or no fat reaches the blood (37), (39).[2] As to the possibility of fat storage along the path of transport, fat is present in the epithelial cells and the intestinal leucocytes for some time after it has ceased to be absorbed from the intestine and it has been found in quite large amounts in the leucocytes in the lymphoid tissue of the intestine (Heidenhain, p. 85) even during fasting. Nevertheless the amount that could be stored in this way is probably not great, and in spite of the lack of evidence of direct absorption into the blood stream the probability is that some at least of the fat is absorbed in that way. The direct evidence presented by Bornstein and Zucker against absorption into the portal system may mean only that their methods of measurement were not sensitive enough.

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[2] Too much weight should not be placed on results obtained by tying off the thoracic duct, for the shock caused by the operation and by the backing up of the chyle must be great, and the failure to demonstrate differences in fat content of the blood during absorption may indicate only that the fat is being removed from the blood as fast as it enters.

TRANSPORT FROM THE BLOOD TO THE TISSUES: *Changes in blood lipoids during fat absorption.* The larger and only traceable part of the fat from the intestine is delivered into the blood stream from the thoracic duct in the form of suspended particles of finely divided pure fat. Traces of soaps or fatty acids have been reported but these are little if any more than could be accounted for by the methods of separation employed. The finely suspended fat in the blood is, as far as can be determined, exactly the same as that in the chyle—particles of about 1μ in diameter, vary even as to size and having a pronounced Brownian movement. During the time that this fat is in the blood and excepting only when little fat is being absorbed, there is to be noted a considerable increase in phospholipoid—"lecithin"—and sometimes also of cholesterol. The increase of lecithin is reported by all investigators who have dealt with the lipoid changes in the blood during fat absorption (40). As far as has been determined, increases in lecithin are most marked in the corpuscles and since fat was increased in them also the inference seems justified that fat is being taken up by the corpuscles and transformed into lecithin (40). The fact that further (unpublished) experiments indicate that the increase in the corpuscles does not always take place need not invalidate the assumption since from work on persistent lipemia (41) it is probable that the lecithin soon passes from the corpuscles to the plasma. As regards cholesterol, while most investigators have not found any increase during fat absorption (38), (40), (42), others (43) have noted increases. The differences in findings as regards cholesterol may be due to the fact noted by Iscovesco that changes in cholesterol values generally do not come until late in the period of absorption and so may be missed if the observations have not been continued long enough. Undoubtedly also, when small amounts of fat are being absorbed no changes at all may be noted in cholesterol. Allowing that the increase of cholesterol in fat absorption is a normal event there appears to be a definite sequence in the lipoid changes following the appearance of large amounts of fat in the blood—the lecithin increasing first and then the cholesterol. It is probable that the increase of fat must reach a certain magnitude before lecithin is formed in notable amounts and that lecithin must be similarly increased before cholesterol begins to increase. In persistent lipemia both lecithin and cholesterol are increased along with the fat, the cholesterol to a greater extent than the lecithin (41). In cases of persistent lipemia that have been studied from their beginning throughout their course (41) the same sequence in the appearance of fat, lecithin and cholesterol noted above may generally be observed, fat increasing first, then lecithin and finally cholesterol. At the subsidence of the lipemia, lecithin and cholesterol persist after the fat has gone and of these cholesterol persists longest. Since lecithin is almost certainly a factor if not a stage in fat metabolism its increase following increase of fat is readily explainable, but it is more difficult to account for the increases in cholesterol, since although it does to some extent (about one-third of its amount) form esters with the fatty acids, these are more difficultly saponifiable than the fats (while lecithin is more readily saponifiable) and can for that reason hardly be regarded as important in fat metabolism. Cholesterol and lecithin are known to be antagonistic in many of their relations in the living body and it may be that the increase of cholesterol following that of lecithin is a reaction whose purpose is to offset the injurious effects of an abnormally high concentration of lecithin.

Further evidence that lecithin (phospholipoid) may be of great if not of supreme importance in the transport of fat in and from the blood is provided by the work of Meigs and his co-workers (44) on milk-fat secretion in cows. These investigators found that during milk secretion the difference in the lipoid phosphorus values of the blood plasma before and after passing through the mammary gland is sufficient to provide the *entire* amount of fat secreted in the milk. Allowing for considerable errors in the calculations on which this assumption is based, the fact remains that a large proportion of the milk fat probably has its origin in the phospholipoid of the blood. There is considerable evidence of an indirect nature which may be brought to the support of the thesis that lecithin in the blood is the main form of transport of fat, and the precursor of fat in the tissues, and conversely, that fat in the tissues on entrance into the blood is changed to lecithin. While, as discussed above, it is improbable that lipases are present in the tissues in amounts sufficient to bring about the passage of fat to and from the cells by hydrolysis, all workers are agreed that there is abundant enzyme in the tissues for the transformation of lecithin (esterases, lecithinases). Lecithin is present in relatively large amount in the organ which is known to be most active in fat metabolism, e.g., the liver; also in the heart where a large reserve supply of readily available energy is required, and in the brain where, although the energy exchange is not great, the conditions for the energy transformation must be very exactly adjusted. Lecithin is the only compound of the fatty acids (with the exception of the soluble soaps which are known to be toxic) which is miscible in water and which can therefore readily follow the lines of water transport. These, together with the observation that lecithin is formed in the blood during fat absorption, present a strong argument for the belief that it is the main form in which fat is moved in the animal body.

On the other hand it cannot be denied that fat may be removed from the blood, at least temporarily, in other forms than as lecithin. During fat absorption, and at other times of large fat transport, as in fasting, diabetic lipemia and in phosphorus and other poisoning, the liver is found loaded with fat, mainly as such although the lecithin content of the liver, if not greater at the time, is generally soon increased (45). Fine emulsions of colored or otherwise marked fat when injected into the circulation are found to collect in certain definite places—the liver, bone marrow, spleen and muscles in the order named (46), in which respect the fat particles behave in the same manner as particles of other foreign matter. For this reason a brief review of the factors involved in the removal of finely particulate matter from the blood is desirable. Evans (47) has called attention to a group of endothelial cells, called by him macrophages, which are found in the vascular system at just those places mentioned above where injected fat is found to have collected, i.e., capillaries of the hepatic lobules, capillaries and venules of the spleen, capillaries and venules of the bone marrow, capillaries and venules of the hemal glands, lymphatic sinuses of the lymphatic glands. Other macrophages which are more or less fixed include reticulum cells of lymph glands and similar cells in the splenic pulp and bone marrow which are probably endothelial; also cells not directly related to the endothelium designated variously as clasmocytes or wandering cells. Besides these there are free macrophages—mononuclear cells found in the serous cavities, in the lymphatic sinuses of lymph glands, in the splenic and hepatic cavities but rarely observed in the peripheral blood stream. Recently Simpson (48) in Evans' laboratory has studied these cells experimentally and has outlined conditions under which vast numbers of these macrophages may be set free in certain portions of the circulation. A characteristic feature of the macrophages is the presence of abundant, various sized and often delicate pseudopodia which may cover their surface and which would indicate at once their function in engulfing whatever free particles were attracted to them. The phagocytic nature of many of these groups of cells has long been known and it is easy to demonstrate with certain dyes, colloidal metals, etc. The actual demonstration of the phagocytic activity of these cells toward the finely suspended fat present in the blood during fat absorption has never been made, but from the general observation of Bondi and Neumann (see above) that injected fat may be found in just those places where these cells are known to be abundant, gives a strong indication that they are concerned in the removal of suspended fat from the blood. That lipoids accumulate in these cells has also been known for some time (49), and the parallelism between the distribution of the vital dyes and the fat bodies has been pointed out. Also it is precisely these cells filled with anisotropic lipoids (cholesterol esters) which constitute the xanthoma and xanthelasma cells and it is especially significant that animals fed for a long time on cholesterol suffer a very general conversion of their wandering cells into xanthoma cells (Evans, p. 257). Whether the fat so taken up is passed on from these cells into the tissues or whether it is stored there temporarily to be given up to the blood stream later for transformation into lecithin cannot be surmised, but it is known that dye granules may remain in these cells for a long time.

PASSAGE FROM THE TISSUES: *Fat in the tissues.* Lipoid material exists in the tissues in several forms, of which fat, phospholipoid (lecithin, etc.), cholesterol and cholesterol esters are present in largest amounts and are best known. The phospholipoid has been found to be characteristic of the organ, e.g., always more abundant in the kidney or liver than in the muscles. In different animals of the same species it varies relatively little and although the variation is somewhat greater in different species the values are quite constant. The content does not vary with alimentation (50). A characteristic relation between cholesterol and the fatty acid content of most tissues has also been noted (51). Inanition produces a rise (which may be only relative) in cholesterol in the tissues, especially the muscle and liver (52), and in the lipoid phosphorus (53) especially in the lungs. The fatty acid content diminishes in inanition and the relation $\frac{\text{Fatty acid}}{\text{Lipoid phosphorus}}$ is much below normal and much lower than in any phospholipoid so far isolated, indicating that some of the lecithin present may contain only one fatty acid group instead of two as normally.

The main form in which lipoid material is stored in the organism is however as fat and although there are but few locations in the organism where traces of fat may not be found it is in general laid away in a special tissue—the adipose tissue—which is collected characteristically in definite locations (54). There is a considerable layer under the skin—the panniculus adiposus, around the internal parts—the kidney, filling up furrows in the heart—in various situations beneath the serous coats or between their folds as in the mesentery or omentum, around the joints, in large amounts in the bone marrow, often in large amounts between the muscle fibers and occasionally in the muscle fibers themselves. The adipose tissue always has a copious blood supply and the lymphatics of the fat tissue are in close relation to the blood vessels. The adipose tissue is modified connective tissue which apparently is predestined to act as a fat store from the early stages of development (55). Other portions of connective tissue may be pressed into service as required, developing a special blood supply for the occasion and passing back to ordinary connective tissue again when it gives up its fat, which is not the case with true adipose tissue.

Fat may be deposited in many cells other than the adipose tissue—cartilage, liver (especially during fat absorption from the intestine), epithelium and in the muscle fibers. In many of these locations the fat deposits are independent of the nutritional condition of the body whereas those in the adipose tissue vary with it. Greene has made a study of the storage of fat in the Pacific Coast salmon at various times in its migration from the sea to the spawning grounds and his data are of special interest in connection with the storage of fat in muscle tissue (56). In the dark muscle, fat is stored chiefly within the fibers but to some extent between them. In the pink muscle (the great lateral muscle of the body) fat is stored wholly between the fibers. In the constantly active fin muscles there is only a small amount of stored fat which is chiefly between the muscles. After feeding ceases the fat slowly disappears but is never wholly consumed although greatly reduced at the time of death.

The liver is generally regarded as a temporary fat storehouse and there are many observations on record to show that fat accumulates in this organ under a great variety of conditions. Thus during fat absorption (57) the fat of the liver is increased. In the course of poisoning with many substances, e.g., phosphorus, the liver generally becomes loaded with fat. Delayed chloroform poisoning is accompanied by a fatty liver. The condition occurs in chronic alcoholism (58), often in fasting (60) and in diabetic lipemia (59). The frequency with which the liver becomes loaded with fat under such diverse conditions has led Leathes (61) and Hartley (62) to the belief that the liver is an important temporary storehouse for the fat passing out from the tissues and they bring evidence to show that changes preparatory to combustion (desaturation of the fatty acids, combination into lecithin) are brought about there. All workers are not in agreement with them in the belief that the liver is a fat storehouse. Thus Terroine and Weill (63) report that the remarkable constancy of the fatty acid content of the liver and the difficulty of diminishing the content by inanition or of increasing it by hyperalimentation leads them to the belief that the liver is not a storehouse of fat. In a study of the fat changes in the liver in geese under forced feeding, Mayer and others (64) found that it was only in the later stages of feeding when the other fat depôts are full that the liver and blood load up with fat. They conclude that there is nothing in their experiments to show that the liver acts normally as a fat storehouse. Terroine (52) found that the liver preserves its fatty acid content after several weeks of fasting. In hyperalimentation it does not increase except in immature animals. Bell (55), in studies on fat cattle, found fat in the hepatic cells of two moderately fat animals while none was found in the very fat animals. He concludes that there is no relation between the presence of fat in the liver and the nutritive condition of the animal.

Fat is laid down in the cells of the adipose tissue of animals in the same way as has been noted for the epithelial cells of the intestine—appearing first as fine droplets which gradually becoming larger, fuse with each other until the cell is filled with a single large globule of fat. In plant cells which store fat, droplets are never seen (65). The fat is so finely divided and so intimately mixed with the other constituents of the protoplasm that individual droplets cannot be distinguished even with the highest powers of the microscope, and only on germination visible fat droplets are found. It is as though the fat has been present in colloidal form and that flocking out took place only as a result of the swelling produced by germination. This condition is interesting because it is never known to occur in storage cells of animals.

In the laying down of fat in the adipose tissue the cells adjacent to the blood vessels are filled first and the filling extends outwards in all directions, which has also been found to be the case with the liver (55). As to what elements or constituents of the cells are concerned with the laying down of fat, very little is known. Certain granules (Altmann's granules) (66) are always found in cells (55) (intestinal epithelial, adipose tissue, etc.) which are concerned with the storage of fat but too little is definitely known regarding them to warrant extended discussion at this time. The possible rôle of a lipase or esterase in the transfer of fat in and out of these cells has been discussed earlier in the paper.

When the stored fat is required by the organism it is removed from the adipose tissue first and the removal continues until these cells are practically empty, while even in death by starvation the fat content of other tissues may not be greatly changed (67), (52), indicating that the supply of fat in these cells if used at all is being continuously replenished from the blood. Thus Greene (56) found that the fat inside the muscle fibers of the migrating (fasting) salmon is present in all specimens at all stages of the migration and its quantity is remarkably uniform. At the time of death considerable quantities are still present in the smallest fibers. This fat is not observable in these muscle fibers when the animal is feeding but appears only when feeding stops.

As regards the stimulus to the outflow of fat from the stores very little is known other than that it is obviously the result of inanition of the tissues caused either by direct deprivation of food or by poisoning. As to the immediate stimulus, the disappearance of glycogen from the liver (and other tissues) has been suggested (68) and an antagonism between glycogen and fat in the tissues has been suspected. Extensive bleeding has been found to cause an outflow of fat from the tissues of certain animals (41) but this again may be the result of inanition due to anemia.

Effect of transport of fat on the blood lipoids. The outflow of fat from the tissues into the blood may or may not cause an increase in the blood lipoids depending on the balance between the inflow from the stores and the outflow to the tissues in which fat is being utilized. If inflow and outflow are equal, the blood lipoid level remains constant. If the inflow is greater than the outflow, due either to flooding the organism with fat beyond its capacity to deal with it as in the lipemia produced by bleeding and also that occurring in the early days of fasting, or to diminished power of the tissues to remove fat from the blood as in diabetic lipemia (41), then the blood lipoids increase until sometimes a visible milkiness (lipemia) is produced. There may however be considerable increases in the lipoids before there is visible lipemia and it cannot be assumed that the increases in this case are due to "soluble" lipoids such as lecithin alone, since cholesterol and fat may both be increased. The form of occurrence of the latter substances then becomes a matter of interesting speculation.

That there is a tendency toward constant values for the lipoids of the blood both in plasma and especially in the corpuscles in the normal animal seems now to be pretty well established (69), and whenever the amount of fat shifted is not too great the blood preserves its constancy of values. After ingestion of considerable amounts of fat the lipoid level and balance is disturbed as noted above. In fasting it may or may not be (70), depending apparently on the nutritional condition of the subject (71), or rather perhaps on the availability of the stored fat, since it is apparently the case that fat may be more or less loosely stored, with the result that the stimulus of hunger may produce an excessive or merely adequate outflow depending on the nature of the storage. It is significant that the increases in blood lipoids in fasting take place only in the first days, and after that the lipoid content remains constant or slowly diminishes till the death of the animal (Daddi, Terroine). Similarly those substances which cause a transfer of fat from the fat stores via the blood such as narcotics, phosphorus, etc., may or may not cause an increase of blood lipoids, probably for the same reasons. Great hemorrhage in certain animals (rabbits) produces an outflow of fat into the blood far greater than the normal mechanism can take care of, and the result is a lipemia. In other animals (dogs) it is apparently impossible to produce a lipemia by hemorrhage, probably for the reason that in these animals the ability to handle fat is much greater than in rabbits, which are not accustomed to much fat in their diet (41). In all cases examined of lipemia lasting for more than a day or two, not only the fat but also lecithin and cholesterol are much above normal.

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THE WATER BALANCE OF THE BODY

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Water balance may be defined as the daily relation between the total amount of water entering the organism through the ingestion of liquids and food and the total output of water lost from the body by way of the kidneys, bowels, lungs, and skin. In the intake must be included the water of oxidation.

For the maintenance of health the intake must be sufficient to maintain the amount of water in the body tissues necessary for maximal efficiency in metabolism and in the execution of other physiologic processes. This involves the removal of waste products and the dissipation of heat. Since heat dissipation constitutes one of the important factors governing the measurement of water in the organism, loss of water by the skin and respiratory tract will be discussed in considerable detail. Although the loss of water by the skin and lungs, on the one hand, and by the kidney on the other, are to a certain extent inversely proportional, the function the water serves in excretion by these channels is different and can not be interchanged vicariously.

Water is essential to the life and function of every living cell. While its importance is and always has been known, its unique properties are not, and have not been so clearly recognized.

In his recent book, Henderson attempts to give to water due recognition. He says:

> In physics, in chemistry, in geology, in meteorology, and in biology nothing else threatens its preëminence. The physicist has, perforce, chosen it to define his standards of density, of heat capacity, and so forth, and as a means to obtain fixed points in thermometry. The chemist has often been almost exclusively concerned with reactions which take place in aqueous solution, and the unique chemical properties of water are of fundamental significance in most of the departments of his science. . . . . The action of water now appears to be far the most momentous factor in geological evolution. The meteorologist perceives that the incomparable mobility of water, which depends upon its peculiar physical properties and upon its existence in vast quantities in all three states of solid, liquid, and gas, is the chief factor among the properties of matter to determine the nature of the phenomena which he studies; and the physiologist has found

> that water is invariably the principal constituent of active living organisms. Water is ingested in greater amounts than all other substances combined and it is no less the chief excretion. It is the vehicle of the principal foods and excretory products for most of these are dissolved as they enter or leave the body.

In closing the chapter on water, Henderson goes on to say:

> Water, of its very nature, as it occurs automatically in the processes of cosmic evolution, is fit, with a fitness no less marvelous and varied than that fitness of the organism which has been won by the process of adaptation in the course of organic evolution. If doubts remain, let a search be made for any other substance which, however slightly, can claim to rival water as the *milieu* of simple organisms; as the *milieu intérieur* of all living things, or in any other of the countless physiological functions which it performs either automatically or as a result of adaptation.

Water constitutes more than 70 per cent of protoplasm, the structural basis of organic life. Hence it follows that water is of the greatest significance to life. Indeed by some physical chemists, protoplasm is looked upon as essentially an aqueous solution in which are spread out colloidal substances of the greatest complexity. Although we are accustomed to look upon the cell as the seat of metabolism, it is not so clearly recognized that water constitutes the medium in which the chemical changes of metabolism occur, that as a *milieu intérieur* it is essential to life, and that it is fundamental to practically all physiologic processes.

Rubner early called attention to the fact that in starvation an animal can lose practically all his glycogen and fat, and half his body protein, approximately 40 per cent of body weight, and still live, whereas the loss of 10 per cent of the water content of the body results in serious disorders, and the loss of from 20 to 22 per cent results in death.

The necessity of water in biologic processes is universal. Animals whose habitat is far removed from sources of water are favored with special mechanisms for their protection during water deprivation. Thus the camel not only has a system of stomachs most capacious and effective for carrying its water supply, but it has humps with great stores of fat which furnish large amounts of water of metabolism, a thick coat of fine hair which minimizes water evaporation, and a digestive tract that wastes no fluid, and permits dry stools only. Certain species of the moth,[1] are provided with a most effective mechanism whereby their own supply of water is furnished through metabolism. It has been demonstrated that they may live for long periods in a desiccator on food which contains but from 5 to 10 per cent of actual water and bring forth larvae containing from 50 to 80 per cent of water (Babcock).

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[1] The clothes moth, the bee moth, the four spotted pea weevel, and the Mediterranean flour moth.

In relation to the duration of life, water occupies a position intermediate between food and oxygen; is more vital than the former, and less vital than the latter. Some data bearing on the relative importance of these three essentials is shown in table 1.

THE CONSTITUTION OF WATER.[2] As commonly accepted, the constitution of water is H_2O and the molecular weight 18. That this holds true only under limited and unusual conditions has been proved recently largely through the work of Röntgen, Armstrong, Eötvos, Ramsey and Shields, and Sutherland. The percentage composition of water, two atoms of hydrogen to one of oxygen, was proved by Cavendish[3] in 1781, although the true explanation of his results was not apparent until the experiments of Lavoisier in 1783 (Bayliss).

The physical properties of water especially its freezing and boiling points, do not coincide with those of a simple compound containing three atoms only of gases with extremely low freezing and boiling points. If it were so constituted, its freezing point would be -150°C. and its boiling point -100°C., in comparison with other similar compounds (Bayliss). Its high critical temperature, cohesion, refractive index, and high surface tension all indicate that its formula is more complex than H_2O. That this is true is widely accepted, at least for temperatures lower than 400°C.

Practically all investigators are agreed that water is a multiple or polymer of H_2O. Röntgen first suggested that all abnormal properties of water could be accounted for qualitatively by assuming liquid water to be a saturated solution of ice in some other form of water. From a study of surface tension energy, Eötvos concluded that liquid water must be a polymer of H_2O. Ramsey and Shields computed from the surface tension that the formula at the boiling point must be $(H_2O)_3$, and in ice $(H_2O)_4$, a result arrived at earlier by Eötvos. Determinations of the freezing point of solutions of water in other solvents point to the formula $(H_2O)_2$. Sutherland has attempted to prove that steam is represented by H_2O, termed hydrol, and ice by $(H_2O)_3$, trihydrol, liquid water being $(H_2O)_2$, a mixture of trihydrol and dihydrol.

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[2] For the discussion of constitution of water see: Millard: Physical chemistry for colleges. New York, McGraw, Hill, 1921, p. 63.

[3] The discoveries of Watt and Cavendish have been discussed at some length by Buckle, *History of Civilization in England*, New York, Appleton, ii, Pt. 2, p. 414.

TABLE 1

Rôle and behavior of oxygen, water, and food

	PHYSICAL STATE	PERIOD BEFORE UTILIZATION	COURSE TO BODY CELLS	RESERVE STORE IN BODY	CONTROL OF INTAKE	PERIOD OF VOLUNTARY ABSTINENCE	DEATH FOLLOWS ENTIRE ABSTINENCE AFTER
Oxygen	Gas	Short—seconds or minutes	Lungs to blood, to cells	Small	Largely automatic	Seconds to minutes	Minutes
Water	Liquid	Somewhat longer, some minutes	Gastro-intestinal tract to liver, to blood, to cells	Considerable	Regulation of water balance. Thirst indicating need	Hours to 18 days	1½ days to 18 days
Food	Liquid or solid	Minutes to hours	Gastro-intestinal tract to liver to blood, to cells. Complicated metabolism	Great	Complex. Varies with types of food. Hunger indicates need	Days to 2 months (50 days)	104 to 117 days for dogs without death

As to the actual number of molecules existing in the various polymers opinions still differ. Bayliss says, "The balance of evidence appears to be that ice is trihydrol, steam monohydrol, and liquid water mostly dihydrol with varying amounts of the two other polymers according to temperature," and he looks upon water as a ternary mixture. From the foregoing it is obvious that water represents polymerized forms of H_2O but that the exact formulae of water in its various phases, ice, water, and steam, have not as yet been definitely determined.

The cause of the marked association of water is ascribed to the extra valence of oxygen. That oxygen is tetravalent at times is accepted by some authorities as proved.

When water combines with salts, the water may be present as hydrol. The degree of complexity of liquid water is supposed, according to Sutherland, to be altered not only by temperature variation but also when it acts as a solvent, a positive ion converting trihydrol into dihydrol and a negative ion causing a reversal of this change (Turner).

WATER INGESTION AND EXCRETION. Normally, water sufficient for the needs is taken daily in the form of liquids and food. Little or no water is absorbed in the stomach. It passes on into the intestine almost immediately, in small spurts occasioned by the contractions of the stomach. Taken without food, the passage of water into the intestine is but a matter of a few minutes or at most of from ½ to 1 hour; 495 cc. of 500 cc. ingested has been recovered from the duodenum within 25 minutes (Von Mering). When taken with food, 2 or 3 hours or more are required. Constriction of the intestinal vessels retards and dilatation increases absorption (Sollmann, Hanzlik and Pilcher). From the intestine it is absorbed rather quickly, the rate depending in part on whether or not it is mixed with food and also probably on the degree of saturation of the body. A small proportion probably remains in the intestine and passes from the body in the stools. It is absorbed from both small and large intestine. After absorption it becomes a part of the water of the organism and plays its rôle in one or another of the various phases of metabolism or in the execution of some physiologic function on the part of some tissue or organ. It may circulate in the blood or lymph stream, it may play a part in any of the numerous hydrolytic or oxidative reactions; it may constitute the water content of the tissues or of one organ as a liquid phase of colloidal solution or as water of hydration, it may serve as the vehicle of transportation; it may be excreted by one organ, only to be carried elsewhere to another field of chemical reaction, or it may act as a lubricant on some glistening surface subject to friction.

It reaches every cell in the organism, and through its properties furnishes the opportunity for chemical reactions, for changes in physical state, and for energy transformation.

Having served these functions it continues to serve in its excretion by way of the kidneys, bowels, lungs or skin, and appears finally in the urine, feces, sweat, visible or invisible, or as moisture in expired air. The proportions excreted by these different channels vary greatly from time to time in the same individual even in health, depending upon the influence of almost innumerable factors, many of which will be considered later.

In health, despite the complexity of the rôle in metabolism, water is ingested and excreted in balanced amounts so that the water content of the tissues remains practically constant and at a level of maximum efficiency. The mechanism works smoothly and automatically. The sensation of thirst indicates the needs of the body, and the sensation of distention of the bladder or bowels the desirability of evacuation.

The maintenance of supply. If water is excreted it must be replaced in order that metabolism may proceed at its maximum efficiency. The need of the body for water is determined largely by environment and metabolism. Water is ingested intermittently as drink or food and ejected intermittently in the urine and stools. But it is supplied continuously to the cells and is also lost continuously by way of the lungs and skin.

Clinical studies of water balance. As ordinarily conducted, clinical studies of water balance are grossly inaccurate, in that they take account as a rule only of the fluids ingested and of the fluids excreted in the urine and feces. Fortunately the weight of the patient is usually followed, which serves as a valuable control.

The accurate determination of water balance is a matter of considerable complexity and cannot be carried out satisfactorily except in a well equipped metabolic laboratory. The table of Soderstrom and DuBois illustrates the various factors entering into consideration of water balance and indicates the need of scientific training and of technical facilities in undertaking its determination (table 2).

A historical sketch of water balance studies. The first reference to the importance of determining the water balance is made by Celsus in *De Medicinae*, in which he says, in a discussion of dropsy, "Nor is it improper to measure both the drink and the urine; for if more fluid is excreted than is taken, so at length there is hope of good health." He further quotes Asclepiades as having instituted abstinence for two days in dropsy secondary to malaria. Araeteus, the Cappadocian, noted disturbances in the urinary output in diabetes. He says of it: "Diabetes is a wonderful affection, not very frequent among men, being a melting down of the flesh into urine." However, he left no records of quantitative studies of water exchange. Sanctorius, a friend of Galileo, was the first to attempt comprehensive studies in water metabolism. While the latter was engaged in the creation of the science of physics and mathematics, Sanctorius, through his assistance, was engaged in applying the thermometer and balance, that is, the tools of science to problems of physiology. In 1614, in his book, *De Statica Medicina Aphorismorum*, he published the results of years of investigation on increases and decreases in his own body weight and upon the factors determining these variations. He compared the weights of food and drink taken daily with the weight of the urine and feces discharged. From these data and the variations in the weight of his body he deduced the "quantity of material lost by insensible transpiration through the skin and lung."

TABLE 2

Water balance

	grams	
Water intake:		
Drinking water	300	
In coffee, milk, and soup	580	
In solid foods	720	
From oxidation of 100 grams of protein	41	
From oxidation of 100 grams of fat	118	
From oxidation of 244 grams of carbohydrate	135	
		1894
Water output:		
In urine	750	
In feces	300	
Vaporized through skin and respiratory tract	700	
		1750
Plus balance to body		144
Gain in body weight		100

Only when science had advanced to the stage where the nature of the chemical processes concerned could be appreciated (1840–1850) were more comprehensive studies of metabolism undertaken. Then in rapid succession came many fundamental investigations dealing with general metabolism including that of water, which were followed some time later by the important studies of water loss of Pettenkofer and Voit, and Rubner, and Wolpert. Within very recent years have come the more exact calorimetric studies of Atwater and Benedict, of Benedict and Carpenter, and of DuBois, and the ingenious study of Lombard of the weight loss each minute by transpiration.

THE WATER INTAKE: *Water and beverages.* Generally speaking more water is ingested than is required by the economy. The chief source of the fluids of the body is water per se, large quantities being consumed also in the form of beverages, such as tea, coffee, cocoa, milk, carbonated waters, and spiritous liquors. Their ingestion results in part from appetite and habit rather than from true thirst. The rôle of age and several other factors will be discussed later.

Water and solid food. More water is ingested in food than is generally recognized. In an ordinary diet as much as a liter of water a day may be taken in the form of so-called solid foods, taking into account the water contents of these foods and also the water resulting from their oxidation. In table 3 are tabulated a number of so-called solid foods, their percentage of water, weight of average servings, and their caloric values. From this table has been purposely omitted all food such as soups, custards, and compotes in which the high content of water is generally recognized. The number of foods with water content of more than 50 per cent is certainly striking.

The distribution of food in nature is singularly interesting. In the tropics, where the loss of fluid from the body is excessive, foods rich in water abound. In the arctic regions, fat constitutes a large part of the diet and water of oxidation plays a relatively larger part.

Water of metabolism. The sources of water of metabolism are *a*, oxidation of organic matter in intracellular respiration; and *b*, other changes in the molecular structure of substances entering into the composition of cells and tissues, such as syntheses, concerned in the building up of dextrose into cellulose or starch, or of amino acids into complex proteins.

Magnus-Levy calculated the amount of water formed in the oxidation of foodstuffs as follows:

100 grams of fat give 107.1 grams of water
100 grams of starch give 55.5 grams of water
100 grams of protein give 41.3 grams of water
100 grams of alcohol give 117.4 grams of water

An ordinary mixed diet yields in the neighborhood of 300 grams of water. Magnus-Levy stated that a 2000 calorie diet yields approxi-

TABLE 3*

Water content above 50 per cent

FOOD	WATER	AVERAGE SERVING	CALORIES	FOOD	WATER	AVERAGE SERVING	CALORIES
	per cent	*grams*			*per cent*	*grams*	
Cucumbers	95.4	50	8.92	Cod steaks, fresh	79.7	100	81.32
Lettuce	94.7	25	4.9	Smelts	79.2	100	88.9
Wax beans, canned	94.6	100	17.74	Lobster	79.2	100	83.98
Celery	94.5	50	9.48	Lima beans, canned	78.5	100	79.0
Asparagus, canned	94.4	100	18.56	Macaroni, cooked	78.4	100	91.03
Tomatoes, fresh	94.3	100	23.4	Brook trout	77.8	100	98.25
Tomatoes, canned	94.0	100	23.18	Green corn, canned	76.1	100	100.52
Brussels sprouts, canned	93.7	100	21.02	Potatoes, boiled	75.5	100	88.67
Beef juice	93.0	100	25.67	Halibut	75.4	100	124.62
Whey	93.0	100	27.39	Bananas	75.3	100	101.11
Water melon	92.0	100	29.81	Plums	74.5	100	82.0
Radishes	91.8	50	15.02	Cream, 18 per cent	74.0	100	200.75
Onions, boiled	91.2	100	41.75	Mackerel	73.4	100	142.7
Buttermilk	91.0	100	36.63	Egg, boiled	73.2	50	56.3
Strawberries	90.4	100	40.0	Rice, boiled	72.5	100	112.45
Spinach, cooked	89.8	100	22.06	Cottage cheese	72.0	50	56.3
Beet greens, cooked	89.5	100	25.80	Veal leg	71.7	100	147.24
Peaches	89.4	100	42.34	Liver	71.0	100	132.46
Pineapple	89.3	100	44.2	Sweet breads	70.9	100	191.41
Koumiss	89.3	100	53.15	Tongue	70.8	100	163.5
Beets, cooked	88.6	100	40.5	Chicken, white meat	70.3	100	158.57
Oysters, solids	88.3	100	50.2	Lamb leg, roast	67.1	100	198.81
Squash, canned	87.6	100	51.39	Pork tenderloin	66.5	100	198.39
Milk	87.0	100	71.23	Salmon, fresh	64.6	100	209.24
Oranges	86.9	100	52.7	Salmon, canned	63.5	100	208.47
Egg whites	86.2	32	17.6	Sirloin steak	61.9	100	249.54
Peas, canned	85.3	100	56.8	Bologna	60.0	100	240.35
Apple	84.6	100	64.57	Turkey, white meat	58.5	100	187.43
Boiled oatmeal	84.5	100	63.28	Pork and beef sausage	55.4	100	303.67
Pears	84.4	100	64.9	Sardines, canned	52.3	100	277.51
Haddock, fresh	81.0	100	73.3	Corned beef	51.8	100	276.16
Clams, solids	80.8	100	75.0	Ham, boiled, smoked	51.3	100	290.84
Prunes	79.6	100	80.18	Mutton leg, roast	50.9	100	312.68

* Table prepared by Mary Foley, dietitian of the Mayo Clinic.

mately 240 grams and a 4000 calorie diet, 480 grams of water, or about 12 grams for each 100 calories.

Intracellular metabolic water results in dilution of cell contents, which disturbs the osmotic equilibrium between the fluids within and without the cell, which occasions movement of nutriments by osmosis to these centers of dilution and of water and carbon dioxid in the opposite direction.[4] As a result the water of metabolism plays a unique part and one that cannot be duplicated by water derived from outside the cell. This holds true for protoplasm of both the animal and the vegetable kingdom. Relatively, the water of metabolism forms a large percentage of the water required for vital processes in animals; indeed in some animals it supplies the entire need of the economy over long periods of time. In animals, also, the water demand for the excretion of waste products is large, but in those having little or limited access to water the end products of nitrogen metabolism appear in solid form, that is uric acid, which is relatively non-toxic and the hydrogen content of which is very low. In many of these animals the metabolism is such as to protect them to the uttermost against loss of water. This is especially striking in certain animals in arid districts, serpents, prairie dogs, mice, sheep, birds, and, as already mentioned, camels, and certain varieties of moths (Babcock).

Hibernation. In hibernation animals depend for long periods solely on their water of metabolism. Well nourished and well padded with fat, they enter their long sleep. Metabolism proceeds at an extremely low level, but moisture is constantly given off by the lungs. The fat furnishes the greater part of the water, protein metabolism being at a minimum. In some instances the thick coat of fur, close quarters, and low temperature usually keep water loss by way of the skin at the lowest possible level (Babcock).

The nature of the sensation of thirst. Thirst is the index to the body need of water. It is a sensation of dryness of the mouth and throat accompanied by a desire for water. It develops automatically with the need of furnishing the fresh supply of water to the organism. Like hunger it is a part of the replenishing mechanism of the body, and like hunger it has been the subject of considerable physiologic investigation. Hunger and thirst must be distinguished from appetite, which develops as the result of habits in eating and drinking.

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[4] This view which has been presented among others, for instance by Babcock, presupposes the presence of cell membranes.

No doubt exists concerning *a*, the reference of thirst sensation to the mucous membrane of the mouth, pharynx, root of the tongue, and palate; or *b*, the scantiness and stickiness of saliva and mucous in the mouth during thirst. The latter has been emphasized strikingly by King, a medical officer who studied a troop of cavalry which was lost for 3½ days without water in the torrid "Llano Estacado" in Texas. He states that long before the third day salivary mucous secretions had disappeared from the mouth and that "brown sugar would not dissolve in the mouth."

Dryness of the mouth may result from local or general causes. Among the local causes are breathing hot dry air, prolonged speaking and prolonged chewing of dry food. In public speaking or singing, fear may constitute an additional factor.

The general causes include factors reducing the general fluid content of the body through excessive loss resulting from profuse sweating, diarrhea, polyuria, lactation or from hemorrhage and shock. Two rival theories of thirst have arisen; one ascribes it to a local sensation and the other to a general sensation or tissue thirst.

The chief argument favoring local sensation is the abolition of thirst by the application of local anesthetics to the mucous membrane of the mouth and throat in diabetes insipidus (Lepidi-Chioti and Fubini) and in dogs (Valenti). Against this, Wassilief has argued that swallowing is made difficult or impossible by local anesthesia, which in turn is denied by Valenti. In arguing against it we might also refer to our own experience in which the local application of cocaine, pushed in one case to the point of constitutional toxicity, failed to decrease the water intake in diabetes insipidus. In its favor we might suggest that the sensation of hunger has been proved to be local in origin and that the sensations attending bowel and bladder distention are also local in character.

The view that thirst is a general sensation is stated by Schiff: "It arises from a lessened water content of the body, a condition from which the whole body suffers. The local reference to the pharynx, like the local reference of hunger to the stomach, is due to association of experiences. Thus the feeling of dryness in the mouth, although it accompanies thirst, has only the value of a secondary phenomenon, and bears no deeper relation to the general sensation than the heaviness of the eyes bears to the general sensation of sleepiness."

The evidence on which the theory of general sensation is based consists of *a*, the abolition of thirst in dogs through the injection of water into the veins (Dupuytren, Orfila); and in a patient suffering from hydrophobia (Magendie); *b*, the striking thirst and the failure of ingested water to relieve it in dogs with open gastric fistulas, an experiment devised by Claude Bernard. He says, "As the animal became thirsty, it would drink until fatigued, and when rested it would begin again; but after the fistula was closed, drinking quickly assuaged the desire for water;" *c*, dogs continue to drink water after the section of the glossopharyngeal, lingual, and vagus nerves on both sides (Longet); and *d*, increase in osmotic pressure occurs in thirst as indicated by the freezing point in conditions naturally accompanied by thirst (Mayer).

According to Cannon these findings do not necessarily indicate that the sensation of thirst is general. Each is capable of interpretation other than that suggested by the investigator, thus: *a*, the abolition of thirst by parenteral administration of water may result in more copious secretion of saliva which, acting locally, relieves thirst; *b*, Bernard's interpretation has been challenged by Voit, who points out that the drinking of water under these conditions moistens the throat only temporarily and does not compensate for decreased salivary secretion resulting from depletion of the body of water; *c*, the continuous drinking after section of the nerves to the mouth may be due to habit or appetite, even if all the branches of the nerves are severed, which is usually not the case (Voit); and *d*, increase in osmotic pressure in thirst does not arise until after two or three days of water deprivation, at which time animals with slightly hypertonic blood will drink salt solutions with apparent relief, while dogs with markedly hypertonic blood drink hypertonic salt solution repeatedly without relief (Wettendorff).

Cannon believes that "in each condition a general bodily need has arisen from a lack of essential bodily material and is signaled by a well-defined thirst. In each the testimony of ingenious persons regarding their feelings has been carefully set down and then explained away." He calls attention to the fact that animals living on land have special buccal glands, while those living in water do not, and cites the work of Bidder and Schmidt in which, following the ligation of all the salivary ducts, the animals were always eager for water. He further relates personal experiences: *a*, mouth breathing resulting in sensation of dryness; *b*, the influence of prolonged water restriction decreasing the salivary output and its immediate increase following water ingestion; and *c*, the decrease in salivary secretion following the loss of 500 cc. of body fluid as sweat. In each instance the relation between the decrease of salivary flow and the sensation of thirst was quite clear.

Thirst, according to Cannon, is a local sensation resulting from local dryness of buccal and mucous membrane, due to a decrease of the secretions from the salivary glands which in turn is dependent on diminished supply of fluid furnished these glands by the body because of its depletion in water. Lack of water secreted by the salivary glands is the cause of the local sensation of thirst, and thirst indicates the need of replenishment of fluid in the body.

RÔLE OF WATER IN THE ORGANISM. *Intermediate water exchange.* Only one phase of this subject is presented by way of illustration. Much fluid after absorption finds its way back into the alimentary tract. In fact, the quantity entering the intestines as digestive fluids, far exceeds that taken by mouth.

The amount of these secretions is large. An approximation of the daily excretions in a man of average size is shown in table 4.

TABLE 4

SECRETIONS	AMOUNT	SPECIFIC GRAVITY	AUTHORITY
	cc.		
Saliva	1500	1.002	Bidder and Schmidt
Gastric juice	2000 to 3000	1.006 to 1.014	Bidder and Schmidt
Bile	300 to 500	1.026 (?)	Pfaff and Balch
Pancreatic juice	500 to 800	1.0075	Wohlgemuth
Succus intericus	3000		Pregl

From this it appears that the amount of fluid excreted into the intestine daily is from 7,500 to 10,000 cc. or from two to three times the amount of fluid ordinarily ingested by mouth and from three to four times the amount ordinarily excreted as urine or about twice as much as the total volume of the blood.

As the contents of the intestine pass downward, reabsorption of fluid takes place, the amount remaining in the feces rarely exceeding 200 cc. each day.

Although considerable is known concerning the influence of certain factors on the amount and composition of these various digestive fluids, the subject will not be discussed here.[5]

Water and heat regulation. Water regulates the temperature of the environment and the organism. This is made possible through its unique thermal properties. The specific heat of water is the highest known for any substance, solid or liquid, with one exception. The latent heat of vaporization of water is the highest known, while the latent heat of cooling, is next only to that of ammonia. The latent heat of vaporization is of universal significance in relation to dissipation of body heat because evaporation occurs at all temperatures. The amount of vapor that air can hold when in contact with liquid is variable, is dependent on temperature and pressure, and is greatest for fluids whose latent heat of vaporization is greatest. Consequently more water can vaporize than any other substance. Heat conduction of water, although low as compared with that of some metals, is yet the greatest known for any liquid. Hence water serves best of all liquids in heat conduction in and away from the body. It is the most ideal buffer for heat in existence, that is, it exerts unparalleled resistance to heat or cold before changing its temperature or its physical state, while in addition its high latent heat of vaporization is of constant value to the organism in the dissipation of heat.

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[5] According to Mathews, the duodenum excretes a large quantity of an alkaline albuminous juice, 50 cc. being collected in one experiment within 2 hours in a dog weighing 5 kgm.

Water, by virtue of mobility is readily shifted so as to meet the constantly changing needs in relation to heat regulation in the body. By virtue of its stability, it places its thermal properties at the disposal of the organism for this important function. Besides, of all liquids, it is the most available.

Water, then, regulates heat distribution and dissipation through its mobility and its ideal thermal properties: *a*, high specific heat, which favors storage; *b*. high caloric demands for its evaporation, which permit a rapid elimination of heat; and *c*, high heat conductivity which provides rapid equalization of heat within the tissues of the body (Barbour). The regulating mechanisms in regard to its dissipation are: *1*, the vasoconstrictor center and the vasoconstrictor fibers in the skin; *2*, sweat and the sweat centers and nerves; and *3*, the respiratory center. The nervous mechanism is responsible for the mammal being homeothermic rather than poikilothermic, and destruction of this mechanism results in its conversion from the former into the latter state.

Physico-chemical properties of water. No less unusual and important than the thermal properties of water are those dealing with physical and chemical processes which take place when other substances are brought into contact with it; processes involving solvent power, ionization, hydration, imbibition, and surface tension. By virtue of its dissociating power water may acquire catalytic activity. It is very questionable whether water ever exists in the organism as such; it is present in the form of a complex salt solution and as such conducts electricity and does not act as an insulator as would free water.

Solvent properties. In the organism water is the universal solvent, but even the process of solution of one salt in water is not entirely clear. However, it is evident that dissociation occurs and that in solution the molecules of a substance are free to manifest the effect of energy due to their movements. The process of solution is one of dispersion, similar in kind to that occuring in colloid solutions but differing as to the degree of subdivision and fineness of the dispersoids.

When one considers the number and character of electrolytes, non-electrolytes, colloids, and gases occurring in the blood and urine, the solvent action of water becomes apparent. Although all these substances are freely soluble in water, the majority of them are not soluble in other liquids, not even in alcohol.

The water content of the tissues. Speaking generally, the amount of water in the body is fairly constant. It varies somewhat with age. The fetus contains a great deal of water; the younger the fetus, the more the water (Fehling). At birth the amount of water in the organism is relatively small to judge from the water content of the blood. It increases at about six months at which time aging begins (Lederer). In old age the tissues are tough and appear to be dry, but according to Ranke, the water content is increased to 81.2 to 84.8 per cent instead of 75 to 80 per cent.

Fat alters the percentage content of water in the body, "generally about twenty to twenty-two parts of protein are soaked in and swollen with seventy-eight to eighty parts (four times as much) water. Fat enters into the interstices of the protoplasm as a dry and waterless mass, neither driving water out from the tissues nor bringing it in to them" (Magnus-Levy).

Engels has studied the water depots of the body following intravenous injection of salt solution. He found that all the tissues except bone became more watery. The muscles, skin, and kidneys take up the greater amount of water, increasing their percentage content of water 3.86, 3.23, and 3.83, respectively. The muscles representing 40 per cent of the body weight take up more than two-thirds of any added water, but continue to act normally in spite of the added moisture.

The state of water in the organism. Water exists in the organism mainly as a salt solution, as the liquid phase of colloidal solution, and as water of hydration. It is of vital importance since its properties and those of the cell determine the amount of the various substances present and their exchange. The turgor of cells is dependent on their water content which in turn is dependent on osmotic pressure or the hydration capacity of the protoplasm itself.

The crystalloids in the saline solution of the body exist in rather fixed concentration as is represented in Ringer's solution: sodium chlorid 0.7 per cent, potassium chlorid 0.03 per cent, and calcium chlorid 0.025 per cent. Locke's solution contains in addition sodium bicarbonate 0.01 to 0.03 per cent and glucose 0.1 per cent. Locke's solution approaches in composition deproteinized plasma and contains both electrolytes and nonelectrolytes.

Blood plasma is composed of water and salts, as in Locke's solution, and in addition proteins, as serum albumin, serum globulin, and fibrinogen, gases, metabolites, enzymes, and special substances such as hormones, and so forth. Of the proteins the albumin forms somewhat more than 50 per cent, globulin somewhat less than 50 per cent, and fibrinogen from 5 to 10 per cent.

Water and protoplasm. For want of space, water and protoplasm and water as a medium of transportation will not be discussed.

Water as a lubricant. In most mechanical devices involving motion, lubrication is necessary. Numerous surfaces in the organism are subject to more or less constant friction, which would result in irritation were it not for lubrication. Thus moisture prevents the serious consequences of friction in the pleurae, peritoneum, joints, and eyes. In the body all surfaces subject to friction automatically control their own lubrication through the excretion of fluid on the surface involved. In most instances water plays the major part.

WATER OUTPUT. *Factors determining the relative loss of water by various channels.* The total output of water is determined by the total intake. The relative output by the various channels differs widely in different individuals and in the same individual under varying conditions. An illustrative water balance from DuBois has already been presented. Careful studies have also been made by Atwater and Benedict, and others.

The human organism as compared to animals varies greatly in its responses to stimuli, partially because of differences in reactivity. It is a matter of common experience to find one patient with sweat literally dripping down the sides, while the skin of another patient, examined under identical environmental conditions, remains entirely free from moisture. With some persons public speaking is accompanied by marked dryness of the respiratory passages, while with others no difficulty of this kind is encountered. Polyuria results from nervous tension in some individuals, while others under the same external conditions, do not experience it. Thus many imponderable influences play a part in water exchange and in water retention in the body. "And some, when the bag pipes sing in the nose, can scarce contain their urine."

Since so many factors play a part in determining the elimination of water by each channel, no hard and fast rules can be laid down as to the relative amount of water excreted by each. Some of the more important factors affecting these losses will be discussed separately in some detail.

Attention might be called to the fact that the amount of water lost by the feces is the smallest and ordinarily subject to least change, while the amounts lost by the kidneys and skin are greatest and subject to the greatest variations. Roughly speaking, the amount of fluid lost by these channels tends to vary inversely, other things being equal.

Heat is lost to the organism through *a*, urine, feces and saliva, which are expelled at body temperature; *b*, expired air (air enters the body at environmental temperature and humidity and leaves it, saturated and at a higher temperature); *c*, evaporation from the skin of sweat and invisible perspiration; and *d*, radiation and conduction from the skin. Under certain conditions heat loss occurs almost entirely (98 per cent) by way of the skin as may be seen in table 5.

TABLE 5

CHANNELS	PER CENT	CALORIES
Urine and feces	1.8	48.0
Expired air in warming	3.5	8.4
Vaporization of water from lungs	7.2	18.2
Evaporation from skin	14.2	36.4
Radiation and conduction from skin	73.0	1792.0

In the calorimeter 24 per cent of the total heat production ordinarily is lost by vaporation. As environmental temperature increases and conduction and radiation become insufficient, the evaporation of water is of ever increasing importance. At 37°C. heat loss is effected entirely through vaporization. The factors usually affecting heat dissipation are: climatic conditions; environmental temperature, relative humidity and winds; rate of metabolism and clothing. Heat loss is a surface phenomenon and the amount of heat reaching the surface is the fundamental factor. Heat is brought to the surface by blood, that is, water. The cutaneous factors are: the emissive powers of the surface; the evaporation of the surface; and velocity of transportation of heat to the surface depending on the conductivity of tissues and the speed of cutaneous circulation (Hill). Here, we are concerned with heat dissipation only in so far as loss of water enters into consideration.

Water elimination by skin and respiratory tract. Most of the determinations of water loss through the skin and respiratory tract have been made under what might be considered standard conditions, that is, fasting at average mean temperature and humidity with approximately the same amount of clothing. As early as 1866, Pettenkofer and Voit showed that daily water elimination by these channels in a fasting man reached as high as 829 gm. This has been abundantly corroborated by the work of Rubner, Schewenkenbecher, Wolpert, Sudovyen, Benedict and Carpenter, and DuBois. Wolpert found that the hourly production of water vapor calculated to a uniform body weight of 70 kgm. varied from 30.9 to 70.9 grams; the seven subjects vaporized from 56 to 60 grams of water each hour at a temperature of 18°C. with a relative humidity of from 35 to 60 per cent. Under extreme conditions the hourly loss may be increased to as much as 1 kgm.

TABLE 6

	WEIGHT	WATER ELIMINATED EACH DAY	CALORIES LOST IN EVAPORATION
	gm.	*kg.*	*per cent*
Man	70,000	12.6	22.0
Dog	30,000	12.2	20.0
	4,000	11.5	9.0
Guinea pig	550	11.3	6.5

Rubner, utilizing a Pettenkofer-Voit chamber, discovered that water elimination is proportional to body weight and not to body surface, that variations in humidity do not affect heat production, and that an increase in moisture in the air decreases the heat lost by evaporation but correspondingly increases that lost through radiation and conduction.

The relation of body weight to water elimination is illustrated in Rubner's table. (Table 6.)

The most comprehensive study of the question has been made by Benedict and Carpenter utilizing an Atwater-Rosa calorimeter. They found that "the average for 158 days, covering 2150 hours, shows that the insensible perspiration of several healthy men, sitting, lying asleep or awake, or engaged in minor activities, is approximately 40 grams an hour." Soderstrom and DuBois utilizing the Sage calorimeter found that normal men between the ages of 20 and 50 years excrete on an average of 29 grams of water an hour, about 700 grams each day, and state that few men depart more than one-tenth from this amount under standard conditions. As a rule the proportion of water lost by the lungs is smaller than that by the skin.

Methods of determining the elimination. The amount of water lost by way of the skin and respiratory tract is difficult to determine accurately. It can be estimated approximately by taking into consideration the daily weight of the body and the amount of material ingested and excreted, as was done in the beginning of the seventeenth century by Sanctorius and in the twentieth by Lombard. However, the best results are obtained through the use of the direct calorimeter constructed with a view of determining water lost by vaporization. Through establishing an air current and determining the amount of air circulating in the system and its water content on entering and leaving, the water of vaporization can be determined.

But even with such facilities the error in determining the loss of fluid by evaporation is large, at times, and absolute accuracy is rarely attained. For the Sage calorimeter, which is especially well adapted for work of this kind, the error is probably plus or minus 3 per cent. Control experiments such as those carried out by DuBois, in which alcohol checks were employed along with the dripping of known amounts of water, showed the average error in determining the water of vaporization to be as high as 6.7 per cent. DuBois says it is doubtful if the determination is much more than 10 per cent accurate under ordinary conditions. For determining the water lost by the respiratory tract a hood was devised by Zuntz; and for that lost by skin, cabinets and chambers enclosing the entire body to the chin are utilized (Rubner and Nuttall). In the study of the loss of water from localized areas, small air chambers have been attached locally to the surface of the skin.

Factors affecting water lost by lungs and skin (climatic conditions). Climatic conditions are of paramount importance and involve factors of temperature, relative humidity, vapor tension, air currents, radiant heat, seasons, day and night, and clothing. These factors may act independently or collectively, in unison or opposition, proportionately or disproportionately. Such a combination precludes prognostication in any given case and necessitates actual determination of water loss. Separate consideration of the nature of the influence will be given each of these factors.

Temperature. Other things being equal, the higher the environmental temperature, the greater the loss of water by evaporation. The capacity of air for holding water vapor increases with the temperature.[6]

Temperature of air, °C.	−1	4.5	10.0	16.0	21.0	26.5	32.5	38.0
Maximum weight of water absorbed by the air, grains for each cubic foot	1.94	2.85	4.08	5.75	7.98	10.9	14.7	19.7

Rubner studied the water vaporized by animals and found that water elimination in guinea pigs increased as the temperature of the air was raised above 15°C. and in dogs it increased with temperatures above 7°C. As the temperature increases, increased rate of respiration develops. In dogs panting is especially marked in hot weather. Under ordinary conditions in man sweating begins in the neighborhood of a temperature of 37°C. Sweating may cease in extreme heat and heat stroke is then imminent.

Temperature and humidity. Rubner made a careful investigation of water loss in both man and animals. He found that animals give off more water in dry than in moist air, an increase of from 200 to 300 per cent, accompanying a decrease of relative humidity from 69 to 31 per cent. In a man in the chamber, Rubner found the water given off from lungs and skin to be:

TEMPERATURE	WATER ABSORBED BY DRY AIR		WATER ABSORBED BY MOIST AIR	
	Lungs	Skin	Lungs	Skin
°C.	*gm.*	*gm.*	*gm.*	*gm.*
15	16.8	9.5	9.0	
20	17.0	37.1	11.7	3.6
25	18.4	57.0	10.9	13.0

Benedict and Carpenter also studied the effect of relative humidity. They found that at a humidity of 30 per cent, 60 grams of insensible perspiration were lost, at 50 per cent, 26 grams, and in one individual studied over a period of 27 days, there were variations from 23 to 35 grams.

Lyon has determined that the average evaporation from lungs and skin of a large laboratory class, when the temperature was below zero outside and 70°F. inside, was in the neighborhood of 60 grams an hour for each person, or about 1364 grams a day.

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[6] One cubic meter of air saturated with water vapor at 37°C. contains 43.465 grams of water (Smithsonian table).

Air currents. The movement of air is also a potent factor in evaporation of water from the body. Ordinarily it plays an important part under conditions of the natural outside environment. Wolpert found that when the air temperature was between 15 and 35°C., the wind from a fan diminished the water loss from the body; above and below these temperatures, it increased the evaporation. Its influence is intimately connected with evaporation, temperature, and humidity.[7]

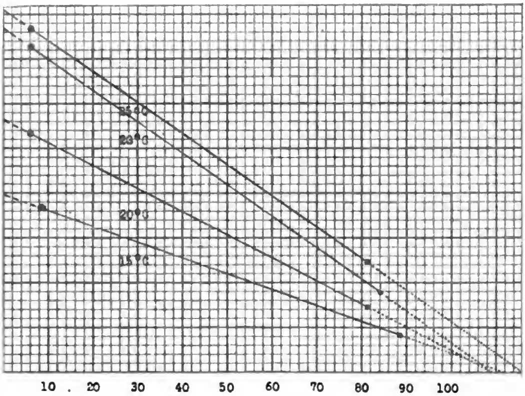

Fig. 1. After Rubner and Lewschew. To show the relation of the loss of water in the form of vapor to temperature and to air moisture. The numerals along the base indicate the degree of air moisture. The verticals represent the water vapor loss in grams.

According to Hill, it appears that the relative humidities around 35 per cent are at least more comfortable than either the extreme dryness or the 50 per cent humidity, which feels quite moist. The influence of temperature and vapor pressure on the amount of water vaporized from the skin is illustrated graphically in the chart taken from Rubner and Lewschew.

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[7] Since at each air temperature above blood heat the bodily gain of heat from the air by conduction increases as the strength of the wind increases, and since the human body cannot perspire above a certain maximum rate, there must, at least on theoretical grounds, be a certain corresponding critical value of the wind velocity which if exceeded must produce a net gain of heat to the body. The

Day and night. Day and night are accompanied by marked changes in the atmosphere. The temperature changes are matters of common knowledge. Relative humidity is subject to variations just as striking. Rubner quotes some figures showing humidity changes by day and night in Vienna. Thus in winter the relative humidity at midday was 75 per cent, at midnight 95 per cent; in summer at midday 49 per cent, and at 6 a.m., 77 per cent. Season also has a marked effect, the average humidity for January being 84 per cent and for April and July 63 per cent.

Effect of food and fasting. The ingestion of food increases the water output especially at high temperature. Rubner found that when dogs were given meat, the water elimination was not affected when the environmental temperature was low but that water elimination was increased markedly when the air was warm. The specific dynamic action of food unquestionably plays a part.

Benedict and Carpenter have shown that a change from a diet poor in carbohydrate to one rich in carbohydrate is accompanied by an increase in weight and by considerable retention of water by the body tissues. Conversely, a change to a diet poor in carbohydrate but rich in fat is accompanied by loss of water from the body. With the caloric intake approximately the same on a diet rich in carbohydrate, there was an average gain of 88 grams of water a day, while in the same subject on a diet high in fat and low in carbohydrate, there was an average loss of 906 grams of water a day. This offers a fertile field for further investigation.

Benedict found that the greatest output of water vapor occurred during the waking hours but failed to find any increase as the result of the

less the temperature of the air exceeds body temperature, the higher will be the critical wind velocity (Hill).

It has been shown that at an air temperature of 45°C. and relative humidity of 40 per cent, a wind of 9 meters per second results in warming, while in a calm cooling occurs.

Huntington has discussed climate in relation to efficiency.

The kata thermometer will probably play a considerable rôle in future investigations of the factors.

Acclimatization or training is an important factor in withstanding the effects of the tropical sun (Shaklee).

ingestion of water. As a rule water lost by the lungs was somewhat less than that from the skin. Benedict and Carpenter have findings in investigations extending over long periods of fasting and have calculated the water vaporized from the lungs as amounting to 9.6 and 10.7 grams each hour and as 40 to 65 per cent of the total amount vaporized.

DAY OF FAST	RELATIVE HUMIDITY	TOTAL WATER VAPORIZED
	per cent	*grams*
1 to 3	52 to 63	23.29
17 to 20	39	15.16
27 to 30	42 to 49	18.20

Laschtschenko showed that there was no increased loss of water by the skin and lungs following the drinking of water up to 2 liters.

Exercise. Light exercise in cool weather has but a slight influence, but hard work in hot weather increases the loss tremendously. Benedict and Carpenter found that with average muscular activity there was a variation from 140 to 276 grams each hour, while at rest under the same environmental conditions the output was from 23 to 79 grams each hour. Obesity tends toward a greater loss of water during work (Schattenfroh).

Wolpert found that a man at rest in still air at 21°C., evaporated 42 grams of water an hour, giving a minimal evaporative heat loss of 576 calories a day. While working at the rate of 15,000 kgm. an hour, he evaporated, under the given conditions, as follows:

TEMPERATURE	RELATIVE HUMIDITY	WATER EVAPORATED EACH HOUR
°C.	*per cent*	*grams*
7	81.0	58.0
13	84.0	70.8
17	87.0	90.4
19	81.0	112.8
25	47.0	230.0

Rest and sleep. Benedict and Carpenter compared the output in rest and sleep. In rest the loss for each kilogram was 0.61 gram an hour with a variation of from 1 to 0.42 gram, while in sleep, the average loss was 0.48 gram with variations from 0.36 to 0.68 gram. The average value in rest for each square meter of body surface was 19.7 grams an hour, with variations from 29.2 to 14 grams while in sleep the average was 15.9 with a variation of from 22.2 to 12.4. During rest the average output is 40 grams an hour, while that in sleep is 32.1 grams an hour.

They further determine the relative amount of water vapor lost from the lungs and skin, utilizing the method of computation devised by Zuntz. This assumes that the inspired air has the moisture content of the air of the room and that the expired air is saturated at the temperature of the body, namely 37°C. Thus by knowing the total ventilation of the lungs it is possible to compute the output of water vapor through the lungs. Zuntz and his associates have determined that for every litre of oxygen absorbed there is a ventilation in the lungs of 21 litres of air, and consequently multiplying the amount of oxygen absorbed by 21 yields the total ventilation of the lungs in liters of air. Although the method is fallacious in some respects, especially during exercise, Benedict and Carpenter believe that it yields fairly accurate and rather constant results during rest. Their results show that in the same individual at rest the ratio of the amount of water vaporized by the lungs and skin is fairly constant and that on an average the amount lost from the skin is approximately 56 per cent of the total, and that this ratio is not materially decreased by food or fasting. Age according to DuBois exercises no great influence on water evaporation for each unit of weight.

Loss OF WATER BY THE SKIN. Water is lost by the skin as insensible and perceptible sweat. The former is continuous as evidenced by the continuous excretion of salt by the skin (Cramer). Sweating is invoked under high environmental temperature around 37°C., but the exact level is influenced by relative humidity, exercise, and certain nervous influences. Loss of water from the skin by transpiration is said to be due purely to physical processes. But this might be questioned on the basis of Talbert's work which demonstrated that volatile acids are excreted continuously during this process. But in patients supposedly devoid of sweat glands losses from the skin up to 800 grams a day have been demonstrated.

Quantity of water lost by way of the skin. Under normal conditions of temperature and humidity this is approximately 500 cc. a day. Nuttall credits Pettenkofer and Voit with 500 cc. Obviously this varies markedly with temperature, humidity, winds, exercise, and clothing. Sweat may vary from an insensible perspiration to as much as 1 litre an hour (Haldane and Priestley; White). According to Flack and Hill as much as 10 litres of water (5800 calories) may be evaporated during a ride in the sun in the South California desert, where the radiant energy reflected from the sand adds its effect to that of the direct radiant energy of the sun.

Water is lost by way of the skin in the absence of sweat glands. Such cases have been described by Guilford, Tendlau, Loewy and Weckselbaum, Christ, and Goeckerman. Loewy and Weckselbaum studied the amount and the mechanism of water lost as insensible perspiration and found in three patients that the amount was normal, 800 cc. each day. Tendlau demonstrated in his patient inefficiency in the heat regulating mechanism, an increase in temperature following the ingestion of milk or direct exposure of the body to the rays of the sun. This patient was studied also by Zuntz who noted increased rate and depth of respiration at all times. In order to work effectively in the sun-light the patient found it necessary to moisten his shirt with water.

Temperature and onset of sweating. Visible sweating appears usually between 30° to 37°C. Von Willebrand observed it in one subject at 30°C. and in another at 33.5°C. Subsequent to the appearance of sweat the local temperature may decrease. "The temperature of the naked human skin," says Aron, "if exposed to the sun, rises quickly to 36°C., maximum 37°C. Sweat breaks out then and the temperature falls." For example, the skin surface temperature of the forehead may rise to 41°C. and sink to 35°C.

Influence of relative humidity. Von Willebrand found that with a relative humidity of 40 to 50 per cent and a temperature of 12°C., 10.5 grams of water an hour were lost by the skin; at 18°C., 13 to 18 grams and at 28°C., 26 to 27 grams. He believes that the amount vaporized gradually mounts with temperature increasing above 12°C. until sweating begins.

For average clothed persons resting in calm air, a temperature of 25° to 26°C. with a relative humidity of 60 per cent occasions sweating With 22 per cent relative humidity, the sweating point is nearly 30°C. (Rubner).

The skin varies in its local temperature, its sweating capacity, and its sensitiveness to heat and cold. Thus Benedict found that the skin temperature varied from 28.1 in the calf of the leg to 34.7 in the waist (with clothing). In an artist's model, after 2½ hours' exposure (naked), in a room at 14.5°C., there was a difference of 10.6°C., between the highest and lowest parts; at 25.8°C. the difference was 5.4°C.; at 30°C., 4.2°C. The forehead secretes earlier than the arm and its temperature falls earlier and generally lower. According to Loewy, the loss of perspiration for each square metre of surface is greatest from the arms, next greatest from the legs (the extremities yielding not far from 75 per cent of the total), and least from the trunk. The greatest actual loss, however, is from the legs (Lusk).

Temperature and exercise. The effect of a warm and a cold day on the sweating of marching soldiers as the result of a 7-mile march carried out by four soldiers is shown (Pembrey).

AVERAGE INCREASE OF	WARM DAY	COLD DAY
Pulse	6.2 each minute	14 each minute
Rectal temperature	2.52°C.	1.44°C.
Evaporation of sweat	1816 grams	419 grams
Weight of clothes (moisture)	320 grams	27 grams

In the summer a young soldier observed by Zuntz, carrying out six marches of 25 km. each, lost between 2436 (1413 calories) and 3366 grams of water (1952 calories), 335 to 995 grams being retained in his clothes.

According to Zuntz, if the body heat production of a soldier is increased 1000 calories by a march carried out at a temperature of 10°C. (50°F.) in saturated calm air, his water loss will be increased 800 grams; each increase of air temperature of 1°C. will increase the water loss 38 grams; each 1 per cent increase of relative humidity will lessen it 4 grams; and each unit increase of wind (12 unit scale of wind) will lessen it 70 grams.

Sweat and clothing. Clothing influences water loss by the skin but this will not be discussed.

Sweat. Sweat contains from 97.5 to 99.5 per cent water, and is probably the most dilute secretion encountered in the organism. The lack of concentrating power of the sweat glands is in marked contrast to the concentrating power of the kidneys. Perspiration has an acid reaction, pH = ± 5.7 (Talbert) and contains, aside from the admixtures of secretions of the sebaceous glands, inorganic salts, chiefly chlorids, and traces of phosphates and sulphates (Kast), and organic compounds of which urea constitutes more than 50 per cent, with smaller amounts of urates, creatinin and etherial sulphates, amino acids, and traces of other metabolic products. The amount of sweat excreted each day varies tremendously with habits, habitat, and climate.

In moderate climates on a daily intake of 3 litres of water the daily loss by sweating during rest approximates 700 cc. for an individual weighing 70 kgm. Heat and exercise markedly increase the amount of sweat. Cramer has estimated from the sodium chlorid left on the surface of the body that more than 3200 cc. are lost daily during a strenuous march in summer weather. By means of dry heat, sweat baths, alcohol or electric cabinets, and so forth, as much as from 0.5 to 1 litre may be lost. Haldane and Priestley refer to two patients who in a Turkish bath, suffered losses as great as 2.3 kgm. of sweat in 2½ hours.

The function of sweat. Sweat, through furnishing water for evaporation, regulates body temperature. This is its chief function. But the sweat glands must also be looked on as excretory organs for water, sodium chlorid, urea and possibly acid radicals. Sweat is a true secretory product and results from the excitation of secretory nerves and is relatively much more independent of blood flow and blood pressure than is the urine. Sweat glands are found everywhere in the skin of man and are very numerous. Cramer has estimated that as many as from 500 to 1900 exist to 1 sq. cm. in some areas of the body.

Sweat in various animals. Men and horses sweat from the entire surface of the body. Dogs and cats show visible sweat only from the hairless surfaces, the soles of the feet; while rabbits, mice and rats do not sweat at all. It is usually contended that dogs do not sweat because they have no sweat glands, but cats and dogs do have such glands in other surfaces than the soles of the feet.

Innervation of sweat glands. The secretory nerves of the sweat glands belong exclusively to the sympathetic nervous system (Langley). In the cat, for instance, the secretory nerves of the sweat glands of the forelegs leave the cord with the fourth to the ninth thoracic nerves; and of the hind legs with the twelfth and thirteenth dorsal and first and second lumbar. They all pass through the sympathetic trunk and then through the brachial or sciatic plexus to the balls of the feet.

The spinal centers are controlled primarily by the thermo-regulatory centers in the mid brain[8] but may also be influenced by other parts of the central nervous system and may be stimulated by various sensory stimuli which often produce sweat only in certain limited portions, as for example, the localized sweating over constantly acting muscles (Meyer and Gottlieb). The sweat during nausea and that due to the stimulation of the cerebral cortex from anxiety or fear are familiar examples of the influence of higher centers of the cerebral nervous system (Winkler).

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[8] The existence of a thermo-regulatory center as such has not been proved.

Heat constitutes the most important and most effective physiologic stimulus for the secretion of sweat and acts upon the higher centers. Sweating is invoked by preventing heat loss and increasing heat production, but as a rule it is evoked most readily by the application of heat. Availability of water for excretion in sweat is an important factor in determining the quantitative response. Local heat renders the secreting glands more amenable to stimulation (Schierbeck), while cooling may prevent it entirely (Langley).

Sweating from drugs. Sweating may be affected by drugs acting centrally or peripherally. All drugs stimulating spinal centers tend to stimulate spinal sweat centers; thus strychnin, camphor, picrotoxin, pilocarpin and ammonium salts increase sweating, but this power is lost after section of the spinal nerves.

Although the innervation of sweat glands, so far as is known, is purely sympathetic (thoracic autonomic), in their pharmacologic reactions, in their insusceptibility to epinephrin and their susceptibility to the autonomic drugs, they behave entirely like organs with autonomic (parasympathetic) innervation. No explanation has thus far been found for the striking exception to otherwise apparently general laws.

Induced sweating by high temperatures. Therapeutically sweating may be induced through the use of high temperatures, either dry or moist, Russian or Turkish baths, dry or moist packs, electric cabinets, hot baths, flannel blankets, and so forth. White found that a few minutes' immersion in a bath at 42.4°C. increased the evaporation of water from his skin eighty times.

LOSS OF WATER BY THE LUNGS. Loss of fluid by way of the lungs is continuous and probably not subject to so great variation as that through the skin. Under ordinary conditions the mucous membrane of the mouth and nasopharynx is always distinctly wet with secretions. The air leaves the respiratory tract saturated with moisture and approximately at body temperature.[9] The mucous membrane of the respiratory tract is susceptible to great changes in regard to turgescence and amount of secretions, as evidenced by the marked sensation of dryness in a hot, dry room.

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[9] This question is discussed in papers by Galeotti and Osborne. The latter claims that the expired air is saturated at temperatures from 32.5 to 33.5°C.

For quantitative measurements of water loss, air may be collected by special devices (Rubner, Zuntz) in the way of cabinets, chambers, or hoods connected in an air-tight manner with the respiratory tract. Rubner found in the resting man at 20°C. that approximately 400 cc. of water, 17 grams an hour (232 calories), escaped through the respiratory channels which is somewhat less than 50 per cent of the total transpiration.

Amount of water lost by the lungs. Benedict and Carpenter, utilizing the computation of Zuntz, found, in a large series of determinations on fifty-three individuals, that the average amount lost by the lungs was 36.3 per cent of the total, with variations from 21 to 51.4 per cent. In sleep the percentage of loss was about 35 per cent.

With the average mean temperature and humidity, Rubner says that an adult gives off water each hour from the lungs: resting, 17 grams; deep breathing, 19 grams; reading, 28 grams; and singing, 34 grams.

The amount of saliva under varying conditions has been studied by Cannon in connection with thirst. Marked effects are demonstrated from mouth breathing, gum chewing, deprivation of water and its administration.

Hill asserts that the water lost during each day by evaporation from the air passages by the resting man can be calculated if the temperature and pressure of the air are taken and the dew point is determined by wet and dry bulb readings.

In the dog the upper respiratory tract is the chief channel of water loss by transpiration. Its influence is indicated by the results following tracheotomy. The rectal temperature of tracheotomized dogs placed in the sunshine rose from 39°C. to 42 and 44°C. The respiration and pulse rate were markedly accelerated, saliva dropped from the mouth, and the mucous membrane became cyanosed.

LOSS OF WATER BY THE KIDNEYS. *The urine.* Usually the urine is regarded as representing the major part of the water loss from the body, and, generally speaking, this is true. However, under unusual conditions of heat and exercise water loss though sweat and evaporation from the skin and lungs may far exceed that of the urine.

Urine represents the end result of the work of the kidney. The mechanism involved in the secretion of urine is not known exactly. The well known theories have recently been discussed by Stieglitz who has added some new evidence relative to the functions of the tubules.

The kidney does not pass substances on merely, for in the excretion marked changes occur in relation to concentration. The comparison of the concentration in some of the more important constituents of the urine and blood are tabulated by Cushny as follows:

TABLE 7

	BLOOD PLASMA	URINE	CHANGE IN CONCENTRATION IN KIDNEY
	per cent	*per cent*	
Water	90 to 93	95	
Proteins, fats, and other colloids	7 to 9		
Dextrose	0.1		
Urea	0.03	2	60
Uric acid	0.002	0.05	25
Sodium	0.32	0.35	1
Potassium	0.02	0.15	7
Ammonium	0.001	0.04	40
Calcium	0.008	0.015	2
Magnesium	0.0025	0.006	2
Chlorids	0.37	0.6	2
Phosphates	0.009	0.27	30
Sulfates	0.003	0.18	60

Cushny's idea of the nature of urine in diuresis should be presented. "The urine in diuresis always approaches plasma in composition more nearly than when the secretion is more moderate." In short all the constituents of the urine are increased in absolute amount for each unit of time during diuresis. But the "no threshold" substances are invariably reduced in percentage, while the "threshold" substances are often reduced in percentage, but may actually rise in some circumstances. As the diuresis passes off, a change in the opposite direction sets in.

The quantity of urine. Generally speaking, the amount of urine excreted is directly dependent on the water intake and inversely proportional to the amount excreted by the other channels of water loss. Ordinarily the kidney excretes daily an amount of water equal to the difference between the intake and that excreted by the skin, bowels, and lungs when the body weight is maintained at a normal level. This amount fluctuates tremendously, in fact, the ability of the kidney to accommodate itself to fluctuations in water and food intake and to environmental influences, constitutes one of its striking characteristics.

This is utilized clinically in determining renal functional capacity.[10] The normal kidney is capable of excreting a small quantity, from 500 to 600 cc. of concentrated urine with a specific gravity of 1.040 or more, or 8 to 10 litres of very dilute urine with specific gravity of 1.001 to 1.002.

Certain great factors are important in determining the daily output of urine. Among these should be considered age, the amount lost by other channels, food intake, salt intake, and finally and most important, the water intake itself.

Age. The urinary output in the early years of life is indicated in Holt's table, his figures being compiled from the findings of several investigators:

Age	*Urine* gm.
First twenty-four hours	0.60
Second twenty-four hours	10 to 90
Three to six days	90 to 250
Seven days to two months	150 to 400
Two to six months	210 to 500
Six months to two years	500 to 800
Five years to eight years	600 to 1200
Eight years to fourteen years	1000 to 1500

Camerer gives findings for the daily urine excretion of boys and girls as follows:

Girls

Years	2 to 4	5 to 7	8 to 10	11 to 14	15 to 18	21 to 24
Output	670	800	980	930	920	1110

Boys

Years	5 to 6	7 to 10	11 to 14	15 to 16	17 to 18
Output	730	940	1040	840	1040

The number of voidings and the average quantity of each is shown in table 8.

The rate and amounts of urine excretion following the intake of definite amounts of water can be found in the papers from Aschenheim and Ohlmann.[11]

Adults. The water intake varies markedly from day to day. The average according to Forster is from 2300 to 3500 cc. These figures are from Münich and include consumption of beer. For this country,

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[10] For details concerning concentration and dilution tests see Volhard and Fahr.

[11] For details concerning urinary output in childhood see Feldman.

Atwater and Benedict found variations in water intake from 880 to 2440 cc. with the subject in repose, an average for 49 days of 2290 cc. and with moderate work from 2225 to 4550 cc., an average for 66 days of 3700 cc. The urinary output varies with the intake within normal limits from 800 to 3000 cc., the average being usually between 1200 to 2000 cc.

TABLE 8

AGE	MICTURITIONS*	AVERAGE QUANTITY VOIDED AT EACH MICTURITION
		cc.
Days, 14 to 30	13.0	34
Months, 1 to 3	14.0	31
3 to 6	20.0	31
6 to 12	16.0	44
Years, 1 to 2	12.0	60
2 to 3	10.0	88
3 to 4	9.0	92
4 to 5	7.5	90
5 to 6	9.3	104
6 to 7	7.1	154
7 to 8	7.8	146
8 to 9	7.0	191
9 to 10	7.3	262
10 to 11	7.0	248
11 to 12	7.5	224
12 to 13	8.3	262

* According to the investigations of Engel and Pfeifer the number of micturitions during the first few months of life may be much greater.

The effects of environment. Other things being equal, the quantity of urine varies directly with the water ingested and inversely with the loss from the lungs and skin. During extreme hot weather, the water intake may be very large and the urine output may be quite small. Breinl and Young have contrasted the urine output in the tropics with that of the European standard. The volume of urine excreted is smaller, the specific gravity higher, and the total nitrogen and sodium chlorid content less in the tropics, as shown by the table giving the average of twenty-five persons some of whom were manual and some sedentary workers:

	IN TOWNSVILLE,* AUSTRALIA	EUROPEAN STANDARD
Quantity	782 cc.	1500 cc.
Specific gravity	1.025	1.015 to 1.020
Freezing point Δ	0.935 to 2.256°C.	0.87 to 2.71°C.
Total nitrogen	10.4 gm.	16.0 gm.
Sodium chlorid	7.0 gm.	15.0 gm.
Phosphates	1.73 gm.	2.0 to 3.5 gm.

* Townsville, Australia, has a latitude of 20 S. and longitude of 147 W.

In hot climates the water balance is usually set at a high level. Hunt says that the fluid intake and output in India is commonly as much as 13 litres each day. In temperate zones, there is a striking difference in the urinary output in summer as compared with winter.

Food and urinary output. Food influences water exchange at least in two important respects, the effects of the metabolites on the water holding capacity of the body, osmotic pressure, salt content, and so forth, and the effects of metabolites on renal function.

Reference has already been made to the effects of diets rich in carbohydrates or fats on body weight and on water output. Rubner calculated the amount of water necessary in tropical weather to yield sufficient water to maintain a properly dilute urine and sufficient sweat to keep the individual cool. This varies with diet. On European diet, 2400 calories a day, 4400 cc. of water are required. On an exclusive meat diet, 7600 cc. of water are required. The effect of types of food on the urinary output is striking. Doctor Adams, of our staff, is studying the effect of diet on the quantity of urine. On each of three days, the patient was given 700 cc. of water with breakfasts rich in carbohydrates (dextrose 1.5 grams for each kilogram of body weight), protein, or fat respectively. On the breakfast high in fat, 27 per cent of the water was recovered in urine within 5 hours, on the diet high in protein, 74 per cent, and on the diet high in carbohydrates, 85 per cent.

Obesity markedly affects water exchange. Oertel's researches show that the quantity of water entering the organism affects the accumulation and consumption of fat. When the water loss is great and the intake small the fat accumulated in the body decreases. Beeler and Fitz have demonstrated however that in one form of obesity, diuresis is induced only with the greatest difficulty. But, in general, it is only with the greatest difficulty that water is held in the body. According to Schattenfroh, the loss of water by transpiration, occurring in fat persons during exercise, is greater than in thin. Lack of training probably plays some part.

Excessive ingestion of sodium chlorid and of sugar results in increased thirst, increased water ingestion, and increased urinary secretion. The ingestion of large quantities of hypertonic salt or sugar solution, results in extreme diuresis; water withdrawal and failure to replenish the excessive loss results in dehydration of the body and development of fever.

Beverages. In addition to the diuretic effect of the contained water, many beverages directly stimulate the renal secretion. Thus the xanthin derivatives in tea, coffee, and cocoa exert a specific stimulating effect on the cells of the renal tubules (Schroeder), and an increased blood flow through the kidney (Loewi). Alcohol, under certain conditions, causes vasodilatation of the kidney and diuresis. Beer is especially effective in this respect.

Control of urinary secretion. Embryologically and histologically, the kidney is not a typical secreting gland. It consists of a large number of units, tubules and glomeruli, and has a relatively large blood supply. The kidney presents within itself the same double capillary arrangement encountered in the portal system; the glomeruli corresponding to vessels of the alimentary tract and the second series around the tubules corresponding to the capillaries of the liver.

The number of units, glomeruli and tubules, is large. In the dog, various investigators have placed the number at from 125,000 to 300,000 depending on the size of the animal (Brodie and Peter); in the cat, 16,000 (Miller and Carlton); in man, 2,000,000 (Schweizer-Seidl). The number is generally in excess of the actual needs, that is, the factor of safety is great as evidenced by the fact that one-third of the kidney tissue will suffice.

The kidney derives its nerve supply from *a*, the semilunar ganglion; *b*, a small branch direct from the splanchnic; *c*, a small branch from the plexuses around the suprarenal body and aorta; and *d*, sometimes a direct branch from the vagus. The splanchnic nerves carry vasoconstrictor fibers. Stimulation results in the arrest of renal secretions (Bradford) and section, in increased flow (Claude Bernard). The vagus, through its action on the heart, exercises a marked influence on renal secretion. Its stimulation in the neck causes a marked decrease of urinary flow, but the presence of fibers directly affecting renal secretions has not been proved. The nerve control is exercised ordinarily through the vasomotor center in the medulla. This may be stimulated directly by asphyxia or anemia of the medulla oblongata, resulting in decrease or arrest of urinary secretion from ischemia of the kidney, or reflexly through the sensory nerves such as the sciatic (Cohnheim and Roy), through cutaneous nerves as in exposure to cold, or through stimuli arising from within the kidney, ureter, or bladder. But it must be remembered that the kidney can function for months in the absence of all nerve supply, as has been shown by Carrel and Guthrie, and Quinby.

Cerebral control is exercised also. The details as to the nature of the mechanism are still lacking. The results of puncture in the floor of the fourth ventricle, Claude Bernard's piqûre (Bernard, Eckhard and Finkelnburg) are familiar. Brain tumors are often accompanied by urinary disturbances, especially those which involve the pituitary gland or its neighborhood. The work of Camus and Roussy indicates that independent of any injury to the pituitary gland, polyuria results from the puncture of the interpeduncular space, and that of Bailey and Bremer indicates that polyuria constantly follows a piqûre injuring the para-infundibular region of the hypothalamus. Polyuria associated with or following epilepsy, migraine, or other headaches, hysteria, and nervous strains suggests strongly the existence of higher centers of control.

The endocrine system also unquestionably plays a part in the control of water balance. This is indicated by the striking effect of the subcutaneous administration of the extract of the posterior lobe of the pituitary in controlling the urinary output, in normal individuals following excessive water ingestion and in patients with diabetes insipidus. Also polyuria results often following extirpation of the posterior lobe of the pituitary or section of the infundibulum. According to Cushing, the pituitary is involved in the control of urinary output through nerve tracts reaching the kidney after passing from the spinal cord to the superior cervical ganglion and posterior ganglionic fibers. Similarly, the thyroid gland is concerned in the metabolism of water as evidenced by changes wrought in a myxedematous patient. On the administration of thyroxin the patient quickly loses weight—largely water—and the dry indurated skin becomes soft and moist.

Some physiologic factors influencing urinary secretion, such as blood flow, blood pressure, etc., will not be discussed.

Physico-chemical factors. The paramount influence in urinary secretion is the chemical composition and physio-chemical state of the blood. Two factors are of great importance, changes in concentration of the colloids of the plasma and permeability of the kidney for substances appearing in the plasma. Decreased colloid content tends to increase urinary output, that is, to "dilution diuresis." The dilution of colloids is most readily accomplished by the intravenous injection of salt solution or Ringer's solution. Such a procedure usually results in calling into play mechanical features, as is evidenced by the increase in the volume of the kidney as recorded by the oncometer. But this does not invariably result (Starling, Cushny). Richards and Plant have shown that the saline diuresis may occur without increase in renal volume or in blood pressure.

Magnus, who conducted classical experiments in salt diuresis, first concluded that it was due to hydremia, but later withdrew this conclusion and ascribed it to specific stimulation of renal cells. In a critical review of the experiments of Magnus, Cushny has reinterpreted his results and finds that decrease in colloid contents of plasma accompanies all instances of diuresis.

EXPERIMENT	NORMAL		DURING DIURESIS	
	Urine in 10 minutes	Plasma-protein	Urine in 10 minutes	Plasma-protein
	cc.	*per cent*	*cc.*	*per cent*
1	1.2	7.33	115.0	3.84
2	1.0	5.94	57.5	3.17
3	1.5	4.43	62.5	2.86

In some experiments when the colloids of the blood are decreased by less than one-half, the urine is increased a hundred fold. Cushny explains this on the basis that the rapid flow of urine through the tubules, that is, flooding, prevents reabsorption and consequently only a small proportion of the excess passing through the glomeruli is reabsorbed, the greater bulk of urine reaching the ureter before absorption. The work of Barcroft and Straub lends support to the idea that lessening the osmotic resistance in filtration is responsible for diuresis, in that the oxygen consumed during the period of diuresis does not exceed the normal amount for the resting kidney. The volume of blood is not responsible, since replacement of colloid by salt solution results in marked diuresis. Knowlton has brought convincing proof, by injecting saline solution with and without colloids and finding that the diuresis is proportional to the free saline solution. Fischer has pointed out that availability of water is important in relation to its excretion and has emphasized the importance of considering the factors affecting the hydration properties of the body tissues. "Free" water only is available for excretion. Meyer and Gottlieb have found that by diminishing colloid content of the blood, urinary secretion can be obtained at as low a pressure as 13 mm. of mercury.

Sodium chlorid diuresis. When sodium chlorid is taken by mouth in excess it may be excreted *a*, in increased concentration but without diuresis; *b*, in normal concentration with diuresis; or *c*, with decreased concentration and diuresis. What happens probably depends on the concentration, at the time, of sodium chlorid and the amount of water in the blood and tissues of the organism. The concentration of other substances is also of importance.

The response to salt intake is extremely important in certain types of nephritis in which the salt and water content of tissues is already disturbed. Additional salt under these conditions usually leads to dropsy and control of salt intake is important in the control of dropsy (Widal and Javal). The recent work of Haldane and Priestley is important in that it shows that water taken by mouth in excess does not result in demonstrable dilution of the blood, whereas following the ingestion of the same quantity of Ringer's solution dilution can be shown.[12]

Water diuresis. Distilled water administered intravenously does not lead to immediate diuresis (Thompson). It quickly leaves the blood stream, owing to the higher osmotic pressure of the cells of the body, which causes it to be taken up by the tissues. Larger quantities lake the blood. Similarly, subcutaneous injection is not followed by diuresis for the same reason (Ginsburg and Cow). Polyuria usually appears within a few hours following the ingestion of water by mouth in excess of from 10 to 15 cc. for each kilogram (Hashimoto).

As already indicated, the most important factor affecting the urinary output is the intake of water. When large quantities of water are ingested by mouth, profuse excretion of very dilute urine follows. As much may be passed in an hour as is ordinarily voided in 24 hours. How is this effected? No adequate explanation has been given. The inference is that water is absorbed; the absorption results in hydremia, which in turn results in diuresis. This appears rational at least, but Haldane and Priestley failed to demonstrate any change in the concentration of the hemoglobin of the blood following the ingestion of water up to 5.5 litres in 6 hours, which resulted in a urinary secretion as high as 1200 cc. in 1 hour, probably the greatest diuresis ever observed in man under normal conditions. They conclude that no demonstrable dilution of the blood accompanied the marked diuresis occurring after the ingestion of huge quantities of water. Priestley, however, in other experiments, succeeded in demonstrating a very slight decrease in the electrical conductivity of the blood and believes that the slight diminution in electrical conductivity indicates very slight decrease in salt concentration of the blood and that the kidney is extraordinarily sensitive to changes in concentration of the blood. He likens the response of the kidney to slight dilution of the blood to the marked increase in ventilation of the lungs resulting from extremely small changes in the carbon dioxid content of the blood. This may be so; however, it has not been proved as yet. Fischer explains diuresis as a result of an excess of free water being brought to the kidney. Cow, failing to find any adequate explanation for the differences in the diuresis from water, when it is given hypodermically and by mouth, has suggested that in the latter case an enzyme from the alimentary tract comes into play. Adolph believes that water held by the tissues is distinct from water freely absorbed from the alimentary tract. Whatever the mechanism may be, this polyuria can be prevented by the administration subcutaneously of a small amount of the extract of the posterior lobe of the hypophysis.

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[12] Ringer's solution practically speaking is optimal fluid.

In diuresis from water ingestion the percentage of urinary solids is largely reduced but the absolute quantities over short periods of time and usually the total excretion tend to increase. The various solids however, do not increase in the same proportion (MacCallum and Benson, Marshall, Carr).

The secretion of urine more dilute than plasma is another matter difficult of explanation. This fact is constantly utilized to disprove Ludwig's theory of urinary secretion and to support the idea of specific secretion of urine. It is difficult to conceive of a urine more dilute than glomerular filtrate (deproteinized plasma) resulting from a mechanism involving filtration of deproteinized plasma and subsequent reabsorption of Ringer's solution by the tubules. Burian believes that the glomeruli secrete a very dilute solution after large amounts of water and Frey that the glomerular solution is unchanged but that the tubules secrete water in addition. Cushny, however, believes that the explanation is not difficult. He states that water is absorbed, enters the blood as a dilute saline solution, and is rapidly carried to the kidneys "where it is filtered off in a form slightly more dilute than the optimal fluid; that the subtraction of the latter in the tubules leaves water containing urea and the other 'no threshold' substances and that such 'threshold' bodies as are above the threshold, potassium, uric acid and almost all of the chlorid, are reabsorbed in the fluid, but some escape." He illustrates this by a hypothetical example, which, however, involves a 10 per cent dilution of plasma which he admits is far beyond the actual dilution ever encountered in the plasma. Thus it is seen that much concerning renal secretion still awaits solution.

Explanations involving the physiology of ordinary diuresis from water must precede those dealing with abnormal or pathologic forms of diuresis. MacCallum has obtained, in man, a diuresis of 20 cc. a minute; Priestley, one at the rate of 1200 cc. an hour from water. Priestley intimates that this rate of elimination results in strain and consequently cannot be sustained. Yet Trousseau records a case of diabetes insipidus in which the patient voided 43 litres of urine a day. Enormous quantities of urine, 15 to 25 litres a day, may be excreted for days, weeks, months or years by persons suffering from this disease without the manifestation of fatigue on the part of the kidney, and without evidence of renal insufficiency. In the primary type of this disease, pathologic conditions cannot be demonstrated. Attempts to demonstrate dilution of plasma by cryoscopy have yielded discordant results,[13] as have also attempts to show increase in the volume of blood (Larson, Weir, and Rowntree). Consequently, we are forced to the conclusion that no adequate explanation has been given for diuresis following the drinking of water, for the polyuria of the diabetes insipidus, or for the antidiuretic effect of pituitary extract.

Water in the feces. Under ordinary conditions the water of the feces rarely amounts to more than 200 cc. in health. Usually it is between 60 and 150 cc. On a vegetarian diet it may reach 300 cc. a day. Occasionally attacks of diarrhea develop, for a day or so, resulting in a doubling or trebling of this amount. But on the whole in health the amount is small and fairly constant.

Water requirements of the body. For continuous health the water intake of the body must suffice to keep the water content at the level for maximum efficiency, from the physiologic viewpoint, irrespective of the loss of water by the various channels of excretion. The important determinants have been presented. This necessitates an intake large enough to replenish the store as losses occur. Additional water is required during childhood for the building up of tissues. Its importance in infancy can be seen from the following data from Reusing.

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[13] It is possible that pituitary extract changes the relative ease with which water passes from the blood stream to the tissues, on the one hand, or to the kidneys on the other, or that it affects the rate of its reabsorption in the tubules of the kidney.

On the breast, the intake the first day is 38 cc. and the urinary output 8.4 cc., while on the eighth day the intake is 338 cc. and the output 208 cc. In artificial feeding, the water intake is usually larger, the amount the first day being 96 cc. and the urinary output 36 cc.; the intake on the eighth day is 530 cc. and the output 406 cc.

There still remains for consideration the effects on the organism of water restriction or deprivation, of water withdrawal from the tissues, and of excessive water ingestion.

THE EFFECTS OF WATER DEPRIVATION. As has been intimated, the body need of water is indicated by thirst. According to McGee, thirst may be divided into five stages:

1. The mouth and throat become dry; a longing for liquid is easily assuaged by ingestion of fluids.
2. The saliva and mucus in the mouth and throat become scant and sticky; the tongue clings to the teeth or to the roof of the mouth; there is a lump in the throat and endless swallowing; this stage is also greatly relieved by water.
3. The eye lids stiffen over the eyeballs which set in a sightless stare.
4. The distal end of the tongue hardens to a dull weight.
5. Delirium develops with visual illusions of lakes and running streams.

Dryness of the mouth is very striking. Reference has been made to King's report on the degree of dryness of the mouth. Suffering was intense and those who survived did so by drinking their own urine or horses' blood.

Thirst is more difficult to endure than hunger. Viterbi, an Italian political prisoner, who died as a result of refraining from food and water for 18 days, suffered but little from hunger after the first day but experienced terrible thirst until the end.[14]

The period that life can be tolerated without water varies tremendously, depending largely on environmental conditions and muscular activity. Thus in the desert, when evaporation is extreme, death occurs as a rule in from 36 to 72 hours. An instance is described in which a Mexican, lost in the desert, walked and crawled 150 miles for a period of 7 days. He sustained life in part by drinking his own secretions.

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[14] According to Hertz, *The Sensibility of the Alimentary Canal*, London, 1911, there comes a time (case quoted) at which neither thirst nor hunger causes distress.

Animals have borne complete starvation from water and food as long as 28 days with recovery. One of Poletayeff's dogs survived 22 days without water with 47 per cent loss of weight, and succumbed in a subsequent starvation only after a loss of 60 per cent body weight. At necropsy, fat was still found in the neck and in the abdominal wall and cavity. A rabbit survived 8 days' complete starvation, and lost 32 per cent body weight. After 7 days it was again starved, and died on the tenth day with 40 per cent loss of weight. Groll has shown that with complete starvation the hemoglobin may increase prior to death 13 per cent in rabbits, 28 per cent in cats, and 18 per cent in dogs.

Keith deprived dogs of water and food over periods of from 2 to 4 weeks. Blood and plasma estimations on these animals showed a definite decrease in the amount of circulating blood. Associated with this diminished blood volume there was an increased viscosity of the whole blood. The viscosity of the plasma may or may not be increased. Partial restoration of blood volume followed the giving of water by mouth. With the addition of food to the water ration the volume of blood appeared to increase more steadily. With this partial restoration of the blood volume, there was a dilution of the previous concentrated blood which was evidenced by a lower viscosity and a decrease in the concentration of the chlorid and nitrogen in the plasma. Some interesting changes were also noted in the hemoglobin and red cell content of the blood during these striking volume changes.

Withdrawal of water from the tissues. In 1899, Crandall described febrile attacks in infancy, occurring in inanition, which he showed to be due to thirst and which disappeared on the administration of water. Finkelstein, in 1908, described fever in children resulting from the administration of hypertonic lactose solution (12.5 per cent) and also from salt and glucose administration, conditions which have since been referred to as salt or sugar fevers. Subsequent workers, Heim and John and Peteri, interpret salt fever as resulting from desiccation and decreased evaporation of water due to the hydropigenous or edema producing action of salt in the blood. Peteri found that the height of the fever attained was in inverse ratio to the body weight. Woodyatt and his collaborators have succeeded in producing glucose fever in adult humans and in dogs through the intravenous administration of concentrated solutions of glucose. In man, difficulty was encountered in eliciting the hyperthermia with single doses of glucose. But they determined that it could be produced readily, provided a period of starvation preceded the glucose administration.

Woodyatt, utilizing his method of constant rate of injection, has produced fever up to 125.6°F. with glucose and up to 111°F. with salt and lactose. He ascribes the hyperthermia to water deprivation, since he has prevented fever by administering water in experiments in which he gave 10 grams an hour for each kilogram for a period of 7 hours. By producing fever with crystalloids, he excluded combustion of sugar as the cause; by preventing convulsions with anesthesia, he excluded convulsions as the cause; and by producing fever in dogs rendered poikilothermic, he excluded action on the heat centers as the cause. Accepting Fischer's conception of "bound" and "free" water, he holds that generally purely physico-chemical processes rob the tissues of free water and disturb the equilibrium between free and bound water. Similarly, he feels that febrile diseases such as typhoid fever and pneumonia may well be the result of free water deficit, due to the abnormal binding capacity of the colloids, and cites the familiar diuresis accompanying the crisis of pneumonia or edema as disturbed water

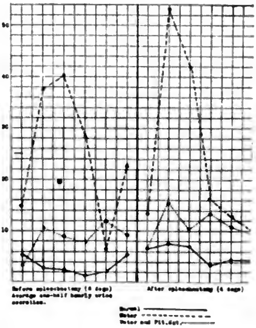

Fig. 2. Average half-hour rate of secretion of urine in dogs under normal conditions, after water, and after water and pituitary extract: (a) before section of the renal nerves (six dogs) and (b) after section of the renal nerves (four dogs).

metabolism. He looks on fever, as Fischer does on edema, as due to physico-chemical changes in colloids resulting in abnormal water binding. Barr and DuBois have pointed out that the relationship between the percentage of calories lost through the vaporization of water and the total heat elimination is the same in patients with fever as in normal controls and in patients with various afebrile diseases.

THE EFFECTS OF EXCESSIVE INGESTION: WATER INTOXICATION. Through thirst the intake of water is regulated to the body needs. As a rule, the amount exceeds the absolute need. Unless the intake is greatly in excess, it is readily excreted by the kidneys and in a less amount by the skin.

In diabetes insipidus, the water balance is set at a level higher than normal. The level can be lowered immediately by the subcutaneous administration of the extract of the posterior lobe of the hypophysis (Von der Velden and Farini). Subsequent to the use of the extract the ingestion of water in the large amounts to which the patient has become accustomed results in marked toxicity. Larson, Weir and Rowntree observed patients who developed headaches, nausea, asthenia, incoördination, marked sweating and, in one instance, mild subcutaneous edema under these conditions. These findings are in accord with those of Miller and Williams. Patients with chronic nephritis and hypertension, to whom they administered water up to 10 litres a day, developed headache, dizziness, restlessness, chills, fullness of the abdomen, vomiting, dyspnea and cramps in the legs, marked increase in weight, and increase in blood pressure.

Water intoxication was produced in dogs by Larson, Weir and Rowntree through the administration of large amounts of water by the stomach tube subsequent to subcutaneous administration of extract of the posterior lobe of the pituitary gland sufficient to prevent the development of polyuria.[15] The ingestion of large quantities of water at hourly intervals, 50 cc. for each kilogram, subsequent to 3 cc. pituitary extract, resulted regularly in the development of the following train of symptoms: asthenia, restlessness, frequency of urination, diarrhea, nausea, retching, vomiting, tremor, salivation, muscle twitching, ataxia, convulsions tonic and clonic, frothing at the mouth, and stupor or coma. Death ensued when water administration was continued after the onset of the convulsions, whereas complete recovery resulted within 12 hours in a large proportion of the animals if no more water was given. Pituitary extract prevents the development of marked diuresis as indicated in the preceding chart.

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[15] The history of the development of our conceptions relative to the rôle of pituitary extract and the function of the posterior lobe is of singular interest. Schafer and his co-workers called attention to the diuretic properties of the extract of the posterior lobe. The work was conducted on animals under the influence of anesthetics. Following this a chemical conception arose involving diabetes insipidus resulting from underfunction of the pituitary (posterior lobe). Subsequently Von der Velden and Farini independently discovered its antidiuretic influence in diabetes insipidus. The extract temporarily removes all the cardinal symptoms of this disease.

TABLE 9

Water intoxication

TIME	WEIGHT	WATER INTAKE	TEMPERATURE	REMARKS
		Dog E804		
	kgm.	*cc.*	*°C.*	
8:45 a.m.	5.7	300	98.0	(After water starvation for 2 days)
9:15 a.m.		300		
9:45 a.m.		300		
10:15 a.m.		300		
10:45 a.m.		300		
11:15 a.m.		300		Salivation, vomiting
11:45 a.m.	6.2	300	98.0	
12:15 p.m.		300		Twitching, vomiting
12:45 p.m.		300		Ataxic
1:15 p.m.		300		
1:45 p.m.	6.4	300	98.0	Convulsions lasting two minutes
1:50 p.m.				Stomach emptied with tube; about 200 cc. fluid obtained
		Rabbit 3		
9:30 a.m.	1.7	100	97.9	Distilled water at body temperature
10:15 a.m.		100		
11:15 a.m.		100		
11:45 a.m.		100		
12:00 m.	2.1			Salivated; ataxic convulsions lasting two minutes
12:15 p.m.	2.1		98.0	
1:05 p.m.	2.1			Found dead

Rowntree has since succeeded in inducing water intoxication in various animals without the use of pituitary extract. In dogs, the administration by stomach tube of 50 cc. for each kilogram of body weight at half-hourly intervals results regularly in nausea, vomiting, salivation, convulsions, stupor, and coma within 4 to 8 hours; death ensues if water administration is continued after the onset of convulsions. Cats, rabbits, and guinea pigs are similarly affected by water (table 9).

This intoxication results from ingestion of ordinary drinking water or distilled water, irrespective of temperature. Although the quantities given are excessive, the amount absorbed is definitely limited.[16] The intoxication is not accompanied by significant changes in body temperature, by edema, or by constant or marked increase in plasma volume. The blood pressure is somewhat increased and may reach as as high as 200 mm. of mercury during the actual convulsions. Intracerebral pressure in one experiment was found to be increased to an amount corresponding to that of 35 cc. of water, and this increase was not in amount proportional to or parallel to the increase of blood pressure.

The convulsions are cerebral in origin and of extreme violence at times; they last from one to ten or fifteen minutes. They may be tonic but are usually clonic in character and they are apt to recur at intervals. In the interim, the animal presents a state of stupor or coma with marked muscular flaccidity, extreme asthenia, and abject helplessness. Hypertonic salt solution administered intravenously after the onset of early evidences of toxicity, prevents the onset of convulsions and coma. Necropsy reveals no gross changes.

[16] The organism possesses some mechanism whereby it protects itself from too great absorption of water. In numerous animals (dogs, cats, and rabbits), following the administration of large quantities of water by the stomach tube, only relatively small quantities of water were absorbed as indicated by the weight.

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THE BIOCHEMICAL JOURNAL

EDITED FOR THE BIOCHEMICAL SOCIETY

BY

W. M. BAYLISS, F.R.S., and ARTHUR HARDEN, F.R.S.

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The subscription price, payable in advance, is 42s net ($8.50) per volume.

CAMBRIDGE UNIVERSITY PRESS (ENGLAND)

AGENTS IN THE UNITED STATES

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO, ILLINOIS

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PHYSIOLOGICAL ABSTRACTS

Monthly, price 5s. net. Annual Subscription, post free, 40s.

This Journal is issued by the PHYSIOLOGICAL SOCIETY, acting in co-operation with numerous physiological organizations in Great Britain, America, and other countries.

The Editor, Professor Halliburton, is assisted by a staff of competent abstractors both at home and abroad.

The Journal aims at issuing promptly abstracts of the papers published throughout the world, in physiological and allied sciences (including Plant Physiology).

A number is published at the beginning of each month.

Volume VI began with the April (1921) issue.

Subscriptions are only taken for April to March of the next year, and are payable in advance.

Back numbers, excepting some which are out of print, may be obtained from the Publishers, or any Bookseller. An extra charge is made for some numbers the stock of which is nearly exhausted. Prices on application.

Binding Cases, price 2s. net, are supplied by the Publishers, who can also arrange for binding; prices on application.

PUBLISHED BY

H. K. LEWIS & CO., Ltd.

136 Gower Street LONDON, W. C. 1

Publishing Office, 28 Gower Place, London, W. C. 1.

SUBSCRIPTIONS MAY BE PAID THROUGH ANY BOOKSELLER

GENERAL PHYS

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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Issued April, 1922

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the Act of March 8, 1879

Made in United States of America

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Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
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PHYSIOLOGICAL REVIEWS

Vol. II APRIL, 1922 No. 2

THE CEREBROSPINAL FLUID

LEWIS H. WEED

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It is obviously impossible within the limitations of this review to present a truly comprehensive account of a characteristic body-fluid such as the cerebrospinal liquid. During the last century many contributions to our knowledge of this fluid have been made, though rather sporadically and with long intervals between publications. Anatomists, physiologists, pathologists and other workers have studied the problems of this fluid; the proper presentation of the many phases of the subject would lead into all of the representative biological sciences. But during the past two decades contributions of a physiological and anatomical nature have resulted in definite enlargement of our conceptions of this fluid which so completely fills the cerebral ventricles and surrounds the central nervous system. It is largely with these more recent advances in knowledge that this review will deal, taking from the older literature only those contributions which have founded the essential bases of the biological processes of the cerebrospinal fluid. It is purposed to omit in large measure the exact chemical, pathological and serological aspects of the subject except as the data from these investigations aid in establishing the fundamental anatomical and physiological phenomena which have to do with this fluid. For in this problem, as in many others, it has seemed obvious that the furtherance of investigations upon function has depended largely upon equivalent advance in anatomical knowledge.

The cerebrospinal fluid, as first effectively described by Magendie (48), is a clear limpid liquid of low specific gravity (1.004 to 1.006), colorless, and of a slight but definite viscosity. When withdrawn during life, the liquid usually contains but few cells per cubic millimeter (less than 10) but in many pathological conditions its cell-content may be enormously increased. Various estimates of the amount of the fluid existing in the cerebral ventricles and about the nervous system in adult man have been published; the computation of 100 to 150 grams given by Testut (65) is probably the most reliable, but because of the complexities of the fluid-bed the figures should necessarily be taken as an approximation.

Chemical examinations have demonstrated that the cerebrospinal fluid contains small quantities of inorganic salts, of protein and of dextrose. The inorganic salts are chiefly sodium chloride and potassium chloride; these occur in the ratio of 17.3 to 1, according to Mestrezat (50), whose monograph is a compendium of analyses of both normal and pathological fluids. The average pH value is given by Felton, Hussey and Bayne-Jones (27) as 7.75, with maximum variations of 7.4 and 7.9. Such chemical and physical characters induced Halliburton (37) to term the cerebrospinal fluid "an ideal physiological saline solution," bathing the neurones and maintaining their osmotic equilibrium.

This liquid, then, of distinctive constitution, unlike other body-fluids (except the aqueous humor of the eye) becomes the subject of review. Definite conceptions regarding its circulation are current, for the evidence today points to a constant production of the fluid, its passage through the cerebral ventricles and thence throughout the subarachnoid space, and its subsequent major absorption into the venous system. Necessarily, however, these conceptions must be subjected to critical analysis, to determine the character and reliability of the data supporting these viewpoints. For this purpose it becomes essential to arrive at some understanding of the anatomical mechanisms involved and to ascertain the functional employment of these structures as pathways for the cerebrospinal fluid. Such a discussion, while in no way comprehensively covering the problems of the cerebrospinal fluid, will present at least in part the phases of the subject which have in recent years been most extensively investigated.

THE SOURCES OF THE CEREBROSPINAL FLUID

The description of the glandular histological structure of the choroid plexuses by Faivre (25) in 1853 marked the discarding of the older concept of Haller and Magendie that the cerebrospinal fluid was a product of the leptomeninges (particularly of the pia mater). Faivre made the first histological survey of these villous projections, showing that the cell coverings were epithelial in nature and that there were indications of secretory activity in these cells. Faivre's observations, supported by similar histological descriptions of hyaline-like inclusions by Luschka (47) in 1855, gave origin to the hypothesis that the choroid plexuses elaborate the greater portion of the cerebrospinal fluid; this has remained the hypothesis upon which most of the investigations regarding the source of this peculiar body-fluid have been based.

The purely histological evidence presented in support of this hypothesis, while suggestive, lacks the element of conclusive proof, though for many years accepted without question. During the past twenty years, however, renewed attempts with finer histological and cytological methods have been made to bridge the gap between intracellular secretion-granules (vacuoles) and the actual production of the liquid surrounding the cells. Thus, Findlay's (28) description of the granular structure of the normal epithelial cells of the plexus, with frequent inclusions of slightly pigmented globules representing the fusion of smaller elements and staining with osmic acid, is quite typical of the purely histological demonstration of secretion. Studnicka (64) obtained somewhat similar evidence of secretion by the cells of the plexus and by the ependymal cells of certain areas. Pettit and Girard (55) found hyaline-like globules, similar to those described by Luschka, in the cells of the plexuses in a comprehensive series of animals but did not feel that they represented secretion-vacuoles. Loeper (46), working on the plexuses in man, described pigmented granules and other granules within vacuoles staining with osmic acid; he stated that he believed that such histological findings permitted him to assert that the cells of the choroid plexuses are glandular. Employing methods of supravital staining in addition to the ordinary histological procedures, Schläpfer (61) concluded that the protoplasm of the cells of the choroid plexus contained "globoplasten" surrounded by a lipoid-like capsule; his histological findings offer but little additional support for his assertion that the choroid plexuses secrete the cerebrospinal fluid. Galeotti's (35) description in rabbit and mouse of three different intracellular inclusions (hyaline droplets, fuchsinophilic granules and small basophilic plasmosomes) afforded evidence of cell-activity by the choroidal epithelium but did not demonstrate the elaboration of the liquid by these structures. The same statement may be made of Francini's (32) differentiation of two forms of secretion-phenomena in these cells—droplets formed in the cytoplasm and granules derived from the cell-nucleus. Engel's (23) demonstration of a fuchsinophilic granule and a basophilic granule staining with methyl green is purely histological evidence of intracellular inclusions but the great variability in position of the granules can hardly be interpreted as indicating different phases in the secretion-process. And there is likewise no conclusive proof of function in Hworostuchin's (41) description of the changes in form of the mitochondria of the choroidal epithelium as showing that the cells play an active rôle in the elaboration of cerebrospinal fluid. Yoshimura (80), in a histochemical investigation of the cells of the plexus, believed that the complicated process of secretion of the cerebrospinal fluid was related to the aggregation of the smaller cytoplasmic granules together into larger vacuoles for discharge. Pellizzi's (54) hypothesis that the epithelial cells of the plexuses secreted granules which increased in size by absorption of the fluid-plasm until extruded, was based on a comprehensive study of the plexuses in vertebrates and may likewise be considered as suggestive support of the general thesis.

It seems clear from the observations just detailed that while the many workers upon the histological structure of the choroid plexuses have described certain granules and vacuoles in these epithelial cells, there is no conclusive evidence that these intracellular structures constitute the intracellular mechanism for the elaboration of cerebrospinal fluid. The difficulty of final demonstration that these granules are discharged into the fluid or are in some way dissolved in that fluid, cannot at present be surmounted.

But fortunately observations of a far more conclusive nature have been made by a combination of pharmacological and histological methods. Cappelletti (9) reported in 1900 that ether and pilocarpine increased the flow of cerebrospinal fluid from an experimental fistula and that atropine and hyoscyamine diminished it. While considering that the differences in vascular reaction to these drugs might account for the phenomena observed, Cappelletti felt that the action of pilocarpine as a general stimulant of gland-activity justified the assumption that there was a true acceleration of secretion of the cerebrospinal fluid. Pettit and Girard (55) extended these observations of Cappelletti by introducing histological examinations of the choroid plexuses in animals which had been given muscarin, pilocarpine, ether, theobromine, etc. The administration of these substances was found to increase the volume of the cytoplasm of the choroidal epithelium, so that the cells became doubled in height. Histological study of these enlarged elements, both in the fresh and in fixed material, showed that the cells were divided into a densely granular basilar zone and a clear apical zone. While the clear apical area was indicated in the resting cell, the rapid enlargement of this zone, under the influence of muscarin, ether and theobromine, resulted in the formation of a distal clear vesicular mass. Such a demonstration of histological change in the choroidal epithelium under the influence of drugs which caused an increased flow of cerebrospinal fluid from a fistula, was believed by Pettit and Girard to prove conclusively that the choroid plexuses possessed a secretory function in the elaboration of cerebrospinal fluid.

Meek (49), repeating the experiments of Pettit and Girard, came to identical conclusions. Muscarin caused no change in the choroidal epithelium in the rat but in the rabbit and guinea pig definite alterations were recorded. Meek stated (p. 300) that "the two things most striking about these modified cells are their great increase in height and the appearance of so much clear space at the distal side of the nucleus."

While the observations just quoted definitely relate the choroid plexuses to the elaboration of cerebrospinal fluid, there is available other substantiating evidence in support of this hypothesis. It had long been realized, from pathological examinations of cases of obstructive internal hydrocephalus, that the cerebrospinal fluid must be at least in part produced by some intraventricular structure. In this relationship renewed attention was directed to the choroid plexuses by the discovery by Claisse and Levy (12) in 1897 of a case of internal hydrocephalus associated with hypertrophy of these intraventricular plexuses. Dandy and Blackfan (17), (18) and Frazier and Peet (33) gave additional support to the general contention when they experimentally produced an internal hydrocephalus by occlusion of the aqueduct of Sylvius. Cushing's (14) observation of an exudation of a clear fluid from a choroid plexus exposed in exploration of a porencephalic defect likewise added suggestive substantiation of the hypothesis. Somewhat more tangible proof of intraventricular elaboration of the fluid was afforded by the writer's (68) demonstration that a definite and sustained outflow of cerebrospinal fluid could be obtained by catheterization of the third ventricle through the aqueduct of Sylvius. The outflow from such a catheter was quite similar in amount to the fluid obtained from a cannula in the subarachnoid space; the finding argues strongly for the belief that the major portion of the cerebrospinal fluid is produced within the cerebral ventricles. But Dandy's (16) later experiments constitute dependable evidence not only that the place of production of cerebrospinal fluid is intraventricular but that the choroid plexuses are the responsible structures. Dandy was able to produce a unilateral internal hydrocephalus by obstructing one foramen of Monro; extirpation of the choroid plexus in such an obstructed lateral cerebral ventricle prevented the development of an internal hydrocephalus. Dandy's experiment furnishes the strongest single substantiation of the hypothesis that the choroid plexuses elaborate the cerebrospinal fluid.

From an entirely different aspect, also, corroborative evidence in favor of the choroidal origin of the cerebrospinal fluid is found in embryological observations of the writer (69). In a study of the development of the cerebrospinal spaces it was shown that the first extraventricular expulsion of the cerebrospinal fluid occurred simultaneously with the first tufting and histological differentiation of the ependymal cells to form the choroid plexuses.

The evidence, then, from histological, pharmacological, pathological and embryological standpoints, surely inclines one to acceptance of the hypothesis that the choroid plexuses of the cerebral ventricles largely elaborate the cerebrospinal fluid. It does not seem justifiable to accept the evidence from any one standpoint as conclusive for many of the observations recorded are corroborative only. Yet the general histological structure of these plexuses, the cytoplasmic inclusions, and the modification of the cell-structure by pharmacological agents offer more than suggestive substantiation of the contention. The pathological studies of cases of internal hydrocephalus, the direct observations of the "sweating" choroid plexus, the embryological relationship between differentiation of choroid plexuses and extraventricular spread of the ventricular fluid, and particularly the experimental investigation of unilateral hydrocephalus, when considered as a whole, present a very strong argument, if not wholly conclusive, in favor of the view that the choroid plexuses are the elaborators of the major portion of the cerebrospinal fluid. It does not seem justifiable to discard, as Becht (1) has done, all of the evidence in favor of this hypothesis as inconclusive. While many of the experimental findings, when viewed as isolated observations, may be explained by other hypotheses, the great mass of data cannot be interpreted on any other hypothesis as satisfactorily. Certain of Becht's specific objections to acceptance of the theory of origin of the liquid from the choroid plexuses have been answered within the last year: Wislocki and Putnam (79) demonstrated by histological methods that in cases of experimental internal hydrocephalus there was absorption of foreign solutions through the ependymal cells lining the ventricles but not through the cells of the choroid plexus. These observations were confirmed and extended by Nañagas (53), who showed by similar procedures that an increased intraventricular absorption of fluid occurred after the intravenous injection of hypertonic solutions of sodium chloride; in no case was there absorption of the fluid by the cells of the choroid plexus. With this evidence in hand, it seems justifiable to disregard Becht's contention that the cell-changes in the choroid plexuses, reported by Pettit and Girard and by Meek, may as properly be interpreted as indicative of absorption as of secretory activity.

But even as a working hypothesis, the choroid plexuses must not be considered to be the sole elaborators of the cerebrospinal fluid. Anatomical evidence presented by the writer (68) indicates that the perivascular spaces also pour a certain amount of fluid into the subarachnoid space, where this fluid mixes with the liquid produced in the cerebral ventricles. Such an addition to the cerebrospinal fluid probably accounts for the reported differences between subarachnoid and ventricular fluids on serological and chemical analysis. The ependymal cells lining the cerebral ventricles and the central canal of the spinal cord may also contribute even in the adult a minimal addition to the intraventricular cerebrospinal fluid.

Although a constant elaboration of cerebrospinal fluid by these mechanisms is indicated, it is not known how large a quantity is produced in any given time-interval but it is not unlikely that the majority of computations are by far too large. Most of the estimates in man have been based on the amount of fluid pouring from subarachnoid fistulae (also cases of cerebrospinal rhinorrhea) where the pressure, against which the fluid is produced, is determined solely by the resistance of the abnormal pathway. The same lack of normal conditions renders unreliable the determinations which are based on the rate of flow from experimental cannulae or fistulae. Estimations of the production of fluid based on the absorption of foreign dyes likewise may lead astray. The evidence indicates, however, that there is a constant though not excessive elaboration of the cerebrospinal fluid; the computations of exact quantities thus far given are of but little value.

CIRCULATION OF THE CEREBROSPINAL FLUID

The cerebrospinal fluid produced largely by the choroid plexuses is poured directly into the cerebral ventricles which are lined by ectodermal ependymal cells. That portion of the fluid formed in the lateral ventricles flows through the foramina of Monro into the third ventricle and thence by the aqueduct of Sylvius into the fourth ventricle. From the fourth ventricle the fluid passes out into the subarachnoid space; there is no evidence that functional communications between cerebral ventricles and subarachnoid space exist elsewhere than in this region.

The exact mode of escape of the ventricular cerebrospinal fluid from the fourth ventricle into the subarachnoid space must still be considered as slightly uncertain. It is possible that the inferior velum of the cerebellar roof in the adult is an intact though functioning membrane, as in the embryo; the existence of a perforation (the foramen of Magendie) in this membrane has been termed an artifact because of the dislocation of structures necessary to demonstrate it macroscopically or because of the shrinkage of tissues in embedding for histological investigation. The greater weight of evidence today inclines to a consideration of the foramen of Magendie as a true anatomical opening in the velum—a break in the ependyma and pia. In support of this conception of a true foramen between fourth ventricle and subarachnoid space may be quoted the observations of Cannieu (8) and of Wilder (78) and especially the developmental studies of Hess (38) and of Blake (6). Blake's conception of the formation of this opening—a shearing-off of the base of a finger-like evagination of the rhombic roof—is rendered more certain by recent morphological studies of this region. The two lateral foramina—those of Luschka—connecting the lateral recesses of the fourth ventricle with the subarachnoid space, seem to have as established a basis for their existence as does the medial. It is through these three foramina—or surely in the region of the inferior tela choroidea if through an intact membrane—that the cerebrospinal fluid, produced in the cerebral ventricles, passes into the subarachnoid space.

From the cisternal dilatation of the subarachnoid space in the region of the medial cerebello-bulbar angle the cerebrospinal fluid slowly seeps downward in the spinal subarachnoid space but passes more rapidly upward about the base of the brain and thence more slowly over the hemispheres, surrounding the whole central nervous system. This movement of fluid is facilitated by impulses transmitted to it by the vascular system; in the spinal region there is also an almost equivalent passage of fluid upward. The subarachnoid space, in which the fluid circulates, is, according to current anatomical descriptions, contained between the arachnoidea and the pia mater. Such a description does not present a proper conception of these fluid-containing channels, for it seems far preferable to consider the subarachnoid space to be intraleptomeningeal. On this basis the arachnoid may be described as the outer continuous membrane, intact and fluid-containing, from the inner surface of which project numerous delicate trabeculae, which merge with the pia mater. The surfaces of the arachnoid membrane and of the trabeculae are covered, as is the inner surface of the dura mater, by flattened, polygonal mesothelial cells. Identical cells also clothe the surface of the brain and spinal cord to form the essential cell-covering of the pia mater. All structures (blood vessels, nerves, etc.) traversing the subarachnoid space are likewise covered by these mesothelial elements. The cerebrospinal fluid, hence, is contained within a completely cell-lined system of continuous yet partially interrupted spaces in the meshes of the arachnoid trabeculae. These meshes are of various sizes, increasing from very fine reticular spaces over the cerebral hemispheres to more widely calibered channels in the cerebral sulci and about the spinal cord, and reaching their maxima in the cisternal dilatations about the cerebello-bulbar angle. In the wide channels of this subarachnoid meshwork the cerebrospinal fluid is obstructed but little in its circulation, but in the small meshes the flow of the liquid is slowed.

Apart from their established function as efficient fluid-retainers, the cells lining the subarachnoid space are of great interest because of their changing morphology under different physiological conditions. The writer (70) first noted that these mesothelial cells phagocyted carbon granules introduced into the subarachnoid space and that when phagocytic, the cells increased in size. Essick (24) then showed that the presence of foreign material (laked blood, granules, etc.) caused these cells to become enlarged, vacuolated, phagocytic and finally detached to form free macrophages of the cerebrospinal fluid.

These mesothelial cells likewise have importance in establishing the relations between the subarachnoid and the perivascular spaces, for there is everywhere in the central nervous system a distinct fluid-containing space about each of the perforating blood vessels. The cells of the pia mater turn inward to form an outer wall of such a perivascular channel while the cells of the arachnoid, covering the vessel as it traverses the subarachnoid space, are likewise continued inward to form an inner cuff of this space. Thus each blood vessel penetrating the nervous system is surrounded by a cell-enclosed, peri-adventitial fluid-channel, which communicates directly with the subarachnoid space. The typical leptomeningeal mesothelial cell of this channel has been identified for variable distances from the surface, dependent upon the caliber of the penetrating vessel. The perivascular fluid-channel, when the mesothelial cell-cuff ceases, continues inward to connect directly with perineuronal spaces about the nerve-cells. These ultimate fluid-spaces are potential in character but under certain circumstances they become easily recognizable in microscopic preparations (Mott (52)). While originally termed "lymphatic" in character, these perivascular channels have no connection with the lymphatic system; they represent however an important accessory fluid-system of the cerebrospinal axis, affording direct pathway, uninterrupted by cell-membranes, between nerve-cell and subarachnoid space.

Thus far mention of the dura mater has been omitted, for it has but slight relationship to the cerebrospinal fluid, the subdural space being anatomically and probably physiologically entirely apart from the subarachnoid. But in one respect the dura mater has importance in the present problem: the areas of penetration of the dense fibrous tissues of the dura by the arachnoid represent points of fusion between the two membranes. The most frequent of these areas of penetration are the arachnoid villi—prolongations of the arachnoid membrane so that the arachnoidal mesothelial cells come to be directly beneath the vascular endothelium of the great dural venous sinuses. Identified in adult man, in infants and in the common laboratory mammals (Weed (66), (67)), these villi are covered by typical arachnoid cells, usually of a single layer but often forming whorls and presenting double-layered coverings. The core of such a villus may be a strand-like network reduplicating the subarachnoid space or a myxomatous ground work simulating the perimedullary mesenchyme. In addition to the true arachnoid villi, which occur in the walls of practically all of the dural sinuses, there are found infrequently prolongations of the arachnoid into the dura in other situations. The arachnoid villi are normal structures; the great enlargement of these in adult life results in the formation of the well-known Pacchionian granulations.

The cerebrospinal fluid, then, circulates everywhere about the central nervous system—in the cerebral ventricles and central canal of the spinal cord and also in the tortuous meshes of the subarachnoid space. These channels are all clothed with a specialized cell, fluid-retaining so that a true circulation of fluid may be maintained. And in the arachnoid villi the circulating fluid comes into close relationship to the great venous sinuses of the dura mater.

ABSORPTION OF THE CEREBROSPINAL FLUID

With the evidence indicating a constant production of cerebrospinal fluid and a circulation of the liquid through the cerebral ventricles and throughout the subarachnoid space, it is not surprising that many investigations should have been undertaken to determine the mode of absorption of the fluid. The experiments performed fall naturally into two groups—the physiological observations to determine whether absorption of the fluid is into venous system or lymphatic trunks and the anatomical investigations to ascertain the exact pathways along which the fluid is absorbed. Because of the great importance of this phase of the subject the evidence will be given in some detail.

Modern anatomical studies of the pathways of absorption of the cerebrospinal fluid were first made by Key and Retzius (43) who injected into the spinal subarachnoid space of a cadaver a gelatine solution colored with Berlin blue. While the pressure used was somewhat excessive (60 mm. Hg.), the anatomical continuity of spinal and cranial subarachnoid spaces was demonstrated, for the whole cranial subarachnoid space was filled with the gelatine-mass. Furthermore, the gelatine was traced into the core of the Pacchionian granulations along the great dural sinuses, and through the cell-membranes directly into these venous sinuses. Many beautiful plates showing this passage of the injection-mass but giving evidence of possible rupture are presented in Key and Retzius' monograph. In addition to this major pathway of absorption, these investigators demonstrated a minor accessory absorption of the fluid into the lymphatic system. Quincke's (56) confirmatory observations, though appearing before the monograph of Key and Retzius, did not antedate their earlier papers on the subject. Quincke injected into the subarachnoid space of living animals a suspension of cinnabar granules and, killing the animals at varying periods thereafter, discovered the granules almost wholly within the basilar and spinal subarachnoid spaces, for the most part held by phagocytic cells. Granules were also found along the venous sinuses in structures which he termed Pacchionian granulations; in the cervical lymph nodes likewise particles of the sulphide were identified.

For several years after these publications, the view of Key and Retzius was accepted as establishing the anatomical pathway of absorption of the cerebrospinal fluid. But gradually with the realization that Pacchionian granulations as such do not exist in infants and in the higher mammals, it was felt that this view was inadequate. For the next two decades practically no work was done upon the subject; then suddenly renewed interest in the problem was made manifest by the publication of several important physiological observations demonstrating a major absorption of the liquid into the venous system and a minor lymphatic drainage.

Reiner and Schnitzler (57) injected saline solutions containing potassium ferrocyanide into the spinal subarachnoid space of living animals and recovered the foreign salt in from 30 to 40 seconds from the blood of the jugular vein. Olive oil injected under similar conditions was identified likewise, though the blood stream was slowed. Reiner and

Schnitzler stated that as Pacchionian granulations do not exist in the animals used, other pathways of absorption must exist. Shortly thereafter Leonard Hill (39) reported that saline solution, colored with methylene blue and introduced into the subarachnoid space, could be traced "straight into the venous sinuses." Within a few minutes (10 to 20) after the injection, the blue was identified in the bladder and stomach; the cervical lymphatics became colored only after an interval of one hour. Following injection of potassium ferrocyanide into the cerebrospinal fluid, Ziegler (81) detected the foreign salt in the posterior facial vein in 10 seconds and only after 30 minutes in the cervical lymphatics. Similarly, Lewandowsky (44) identified sodium ferrocyanide in the urine of animals within 30 minutes after intraspinal introduction.

The experiments of Spina (63), though conducted under abnormally high pressures, likewise add support to the idea of a very rapid major venous absorption and a lesser lymphatic drainage. Cushing's (13) observations on mercury and non-absorbable gases led him to hypothesize a valve-like mechanism for drainage into the venous channel. Both of these observers commented upon the absence of Pacchionian granulations in the higher mammals.

Mott (52) in 1910, from study of the brains of animals subjected to experimental cerebral anemia, suggested a new pathway for the absorption of the cerebrospinal fluid, based on the occurrence of distinct spaces about each nerve-cell, connected through the perivascular channels with the subarachnoid space. Believing that this fluid-system contained cerebrospinal fluid, Mott contended that normally the liquid passes from subarachnoid space into cerebral capillaries. Cathelin (10), without supporting data, assumed that the major absorption of the cerebrospinal fluid was into the lymphatic system; and Goldmann (36), employing subarachnoid injections of trypan blue, likewise tentatively favored this view, though acknowledging the weight of evidence in favor of the major venous drainage.

Dandy and Blackfan (17) in 1913 concluded that the absorption of cerebrospinal fluid was a "diffuse process from the entire subarachnoid space," for with the spinal subarachnoid space isolated from the cranial, they found "a quantitative absorption proportionately as great as from the entire subarachnoid space." The evidence for these statements (18), published in detail a year later, was largely based on the excretion by the kidneys of a readily diffusible dye—phenolsulphonephthalein—after its introduction into the subarachnoid space. A very rapid absorption into the blood stream occurred under such experimental conditions while the lymphatic drainage was minimal in amount and very tardy. After subarachnoid injections of india ink, Dandy and Blackfan were able to find no anatomical evidence of absorption of the carbon granules—an observation in accord with those of Quincke (56) and of Sicard and Cestan (62).

It was with these contributions as a background that the writer (66), (67), (68) began his anatomical studies of the absorption of the cerebrospinal fluid. Critical examination of the preceding work was convincing in demonstrating a major venous absorption and a minor lymphatic drainage of the liquid, but the reliable data were practically entirely physiological. Thus the observations regarding the rapid venous absorption of readily diffusible substances seemed wholly dependable but the anatomical evidence was satisfactory solely in demonstrating that insoluble particles (cinnabar, carbon) did not leave the subarachnoid space in any great amount. The only complete morphological investigations were those of Key and Retzius; these were unsatisfactory because the injections were performed on dead material, the pressures employed were high (60 mm. Hg.) and the injection-mass was a viscous colloid (gelatine) colored with Berlin blue. It was felt that the experimental approach must be such that a subarachnoid injection of a true, isotonic solution of non-toxic foreign salts, capable of subsequent precipitation in situ for histological examination and not diffusely staining cellular material, could be made in the living animal, under pressures not greatly in excess of the normal. With these criteria established, experiments were undertaken with subarachnoid injection of potassium ferrocyanide and iron-ammonium citrate in isotonic solution under pressures but slightly above the normal (130–180 mm. H_2O). Subsequently the central nervous system, enclosed in meninges, was fixed in an acid medium; precipitation of the foreign salts as Prussian blue permitted adequate histological identification of the pathway taken. This method was found to meet all of the standards of investigation set.

The experiments were carried out over periods of several hours in living anesthetized animals, with introduction of the isotonic foreign solution into the lumbar subarachnoid space. The spinal and basilar portions of the subarachnoid space were rapidly filled with the foreign solution but the cerebral portion of the space was not completely injected until the experiment had been continued for several hours. Histological examination demonstrated that the solution had not penetrated any of the cells lining the subarachnoid space; the precipitated granules adhered to the surfaces of the cells but were not within the cytoplasm. The foreign solution was found to have passed directly into the venous sinuses by way of the arachnoid villi into which the precipitated granules could be traced from the cerebral subarachnoid space. These granules, representing the foreign solution, were found within the mesothelial cells covering the villi and the endothelial cells lining the venous sinuses, as well as within the lumen of the venous sinus, thus demonstrating the essential pathway of absorption. In no other place was there evidence of direct passage through a cell-membrane as in the villi. The mechanism of passage of this fluid seemed to be a process of filtration from a point of higher pressure (subarachnoid space) to a point of lower pressure (venous sinus), though the factors of osmosis and diffusion were not excluded. The absorption of true solutions from the cranial subarachnoid space was shown to be a much more efficient and rapid process than was the corresponding absorption from the spinal subarachnoid space. Suspensions of particulate matter were found, in agreement with the observations already recorded, to be retained within the meshes of the subarachnoid space.

In addition to this major venous absorption through arachnoid villi directly into the great dural sinuses, an accessory drainage by way of the lymphatic system was demonstrated. This seemed a much slower, less efficient means of absorption of the fluid, caring for but a small fraction of the total. Such lymphatic absorption was wholly indirect; the fluid reached the true lymphatic vessel only outside of the dura and then by way of perineural spaces.

These anatomical findings, based on a standard of experimentation which approximated the normal, agreed largely with those of Key and Retzius, substituting however for the Pacchionian granulation the normal arachnoid villus. Further observations were made to ascertain the truth of the other anatomical hypotheses ventured for the absorption of cerebrospinal fluid: thus, no structure of a valve-like nature was found in many examinations of serial sections of the great dural sinuses. Mott's theory of absorption by cerebral capillaries was excluded by the failure of the injection-fluid to pass into the perivascular system under conditions approaching the physiological. Dandy and Blackfan's conception of a diffuse absorption by the vessels of the subarachnoid space was likewise found untenable, for in no case were the mesothelial cells covering these vessels penetrated by the foreign solution. The strongest argument in favor of the diffuse process of absorption advanced by

Dandy and Blackfan was that the excretion of phenolsulphonephthalein from the isolated spinal subarachnoid space was proportionately as great as from the whole cranial and spinal subarachnoid space. The technique employed by Dandy and Blackfan involved withdrawal of an equivalent amount of cerebrospinal fluid from the isolated spinal subarachnoid space before injection of 1 cc. of the phenolsulphonephthalein solution. The writer (67) believed that this substitution could not be made in this isolated space without increase in the spinal subarachnoid pressure and escape of the foreign dye into the epidural tissues. By reversing the experiment he was able to show that absorption of phenolsulphonephthalein when introduced into the cisterna magna was as rapid when the spinal subarachnoid space was excluded as with the whole cranial and spinal subarachnoid space functioning. The cranial portion of the nervous system seems, therefore, to contain the efficient mechanisms for the absorption of cerebrospinal fluid.

Since the publication of this work in 1914 there have been other observations regarding the absorption of the cerebrospinal fluid; all accord with this idea of a major venous absorption of the fluid. Thus Frazier and Peet's (33) experiments with methylene blue, isamine blue, trypan red, trypan blue and phenolsulphonephthalein demonstrated the greater importance of the rapid venous absorption and the lesser of the slow lymphatic drainage. And Dixon and Halliburton (21), in the course of their studies of the cerebrospinal fluid, confirmed this general conception of the process, finding no escape of particulate matter from the subarachnoid space but a free and rapid absorption of true solutions. Between the two types there occurred a much slower absorption of colloidal solutions than of true solutions, the larger molecules being absorbed more slowly than the smaller. They concluded, as did also Halliburton (37) that "the fluid probably reached the venous sinuses by way of the microscopic arachnoid villi." And recently the writer, in work as yet unpublished, has repeated certain phases of his original investigation of the pathways of absorption, with continuous observations of the pressures of the cerebrospinal fluid and of the intracranial blood vessels. The results confirm in every way the conception of the mechanism here presented.

Thus it seems fair to assume that the absorption of the cerebrospinal fluid is a twofold process, being chiefly a rapid drainage into the great dural sinuses, and in small part a slow indirect escape into the true lymphatic vessels.

THE PRESSURE OF THE CEREBROSPINAL FLUID

Practically all of the methods of recording the pressure of the cerebrospinal fluid deal with connection of the subarachnoid space to an open or membrane manometer. It is very difficult with any of the procedures to avoid the loss of a few drops of fluid, but by immediate replacement of this liquid and by the use of manometers filled to the estimated level of the fluid, the pressures obtained by these simple methods may be accepted as accurate.

Very few, if any, of the early records of the pressure of the cerebrospinal fluid were sufficiently controlled to permit direct comparison with the data obtained by recent workers. As examples of the variation in the pressures recorded by the earlier investigators, the following determinations may be given: Key and Retzius (43), 162 to 216 mm. H_2O in inspiration and 216 to 270 mm. H_2O in expiration, in etherized dogs; Bergmann (4, 5), 80 mm. H_2O in his first observations and in his second series, 120 to 160 mm. of salt solution in narcotized dogs; Falkenheim and Naunyn (26), 100 to 150 mm. H_2O in curarized dogs; and Leyden (45), 80 to 150 mm. and 100 to 120 mm. H_2O in dogs under morphia. Leonard Hill (39) believed, however, that the intracranial tension might vary from zero to 50 mm. Hg. and that the variations reported by the earlier observers were but expressions of this characteristic of the intracranial pressure.[1] Hill's idea of variability in the normal pressure of the cerebrospinal fluid seemed supported by the observations of Dixon and Halliburton (20), who reported 40 to 70 mm. of salt solution as a rough average of the normal pressures obtained in the dog under morphine-urethane anesthesia. And in a single detailed protocol presented, the pressures at 5-minute intervals ranged as follows: 95, 25, 30, 35, 55, 25, 80, 65, 65, 65, 75, 70, 60, 55, 50, 80, 90 mm. of 10 per cent citrate solution.

The most recent work, particularly by American investigators, has given much better knowledge of the range and variability of the normal pressure of the cerebrospinal fluid. Weed and McKibben (75) reported an initial average of 119 mm. of Ringer's solution in cats anesthetized by intratracheal ether, and an extreme constancy of the pressure of the cerebrospinal fluid under such experimental conditions. Becht (1) found considerable fluctuation in the pressure of the fluid in etherized dogs but of lesser extent than did Dixon and Halliburton; his average pressure of 112 mm. (sodium chloride solution of specific gravity of 1.088) was derived from 39 dogs under intratracheal ether. Foley and Putnam (31) presented a slightly higher average of 127 mm. for the normal reading in animals under ether while the average of 100 animals under various anesthetics was 133 mm. of cerebrospinal fluid; no comment upon the extent of normal fluctuations of the fluid pressure was made. And recently Weed and Hughson (72) have reported an average pressure of 119 mm. of Ringer's solution for 77 cats under ether by Woulfe bottle; the fluctuations in the pressure under the experimental conditions were very slight in extent (11 mm. in an animal under observation for 2 hours).

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[1] Within the limits of the physiological phenomena investigated, intracranial pressure may be considered to be identical with that of the cerebrospinal fluid.

The question of greatest physiological interest in this phase of the general problem is that of the maintenance of this pressure of the cerebrospinal fluid. All of the conceptions of the mechanism for the maintenance of this pressure are based primarily upon the rigid character of the bony coverings of the nervous system. This idea that the cerebrospinal axis is situated within a "closed box," to which the physical laws of such a system apply, was first advanced in 1783 by Alexander Monro (51). Monro believed that the substance of the brain, like that of other solids of the body, is nearly incompressible and is "enclosed in a case of bone," assuring therefore the constancy of the intracranial blood content. The development of this hypothesis by Kellie (42) in 1824 led to wide acceptance, and the Monro-Kellie doctrine with but few alterations has served as the basis upon which the physiology of the intracranial contents has been interpreted. The doctrine was modified by the introduction of the cerebrospinal fluid into the consideration by Burrows (7) under the influence of Magendie's epochal studies; the hypothesis was then formulated by Burrows as follows (p. 32): "the whole contents of the cranium, the brain, the blood and this serum (cerebrospinal fluid) together, must be at all times nearly a constant quantity."

Many physiologists have subjected this Monro-Kelllie doctrine to experimental proof not only in dead but in living animals; while divergent conclusions have been arrived at, the general consensus of opinion expressed in the literature of the last century has been in favor of the hypothesis. And in the hands of recent workers a similar unanimity of expression holds though occasional investigators express doubt as to the accuracy of the premise. Lately, Weed and Hughson (73) have experimentally demonstrated the essential truth of the doctrine that (p. 99) "the bony coverings of the central nervous system constitute within tested physiological limits, inelastic and rigid containers; the ordinary physical laws of a 'closed box' may therefore be applied to the cranium." And with appreciation of the variability in volume of the constituents, the hypothesis may be stated, as was done by Weed and McKibben (76, p. 553): "the cranial cavity is relatively fixed in volume and is completely filled by brain, cerebrospinal fluid and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements."

With the cranium and vertebral column serving as rigid containers, the relation of the intracranial vascular pressures to the pressure of the cerebrospinal fluid requires immediate consideration. Current physiological conceptions of these intracranial mechanisms really date from the work of Leonard Hill (39) who advanced the idea that intracranial pressure is (p. 71) "the same as cerebral venous pressure, and varies in the same direction absolutely as general venous pressure, and proportionately as general arterial pressure." Hill's technical procedures consisted in determing cerebral venous pressure in the torcular Herophili and cerebrospinal fluid pressure in the occipital region.

The emphasis placed by Hill upon this equality of pressure dominated physiological opinion for over fifteen years; it was not until the publication of Dixon and Halliburton (20) in 1914 that contradictory evidence was presented. Using experimental procedures essentially similar to those of Hill, Dixon and Halliburton demonstrated that the cerebrospinal fluid pressure is not identical with that of the dural venous sinus, and stated that the fluid pressure is (p. 153) "influenced passively to a small extent by changes in the arterial and venous pressures but such alterations are insignificant compared with the independent changes in pressure which occur as the result of secretory activity." These investigators also showed that increase in the cerebrospinal fluid pressure produced a passive increase in the cerebral venous pressure but not of the same extent; conversely, alteration of the cerebral venous tension caused similar though not identical alteration in the pressure of the cerebrospinal fluid. Under normal conditions the arterial pressure was found by Dixon and Halliburton to be higher than the intracranial venous pressure which in turn was always higher than that of the cerebrospinal fluid.

Becht (1), employing somewhat similar methods, came to conclusions in some respects at variance with those of Dixon and Halliburton though confirming the general contention of inequality of cerebrospinal fluid pressure and cerebral venous pressure. Becht stated that the cerebrospinal fluid pressure was dependent upon both intracranial arterial and venous pressures, though not identical with either. The data obtained indicated that either the cerebral venous (torcular) or cerebrospinal fluid pressure might be the higher but that usually the former exceeded the latter. Passive changes in the torcular pressure were found to affect the pressure of the cerebrospinal fluid, in the same direction but not to the same extent; but within fairly wide limits changes of the cerebrospinal fluid pressure were not believed to alter the pressure of the torcular.

The observations of these recent workers were all carried out with fairly similar technical procedures, particularly for the determination of venous pressure in the torcular Herophili. The very wide divergences in these normal pressures (13 to 601 mm. in Becht's series) indicate that the method is subject to many experimental defects. Weed and Hughson (74), with these technical disadvantages in mind, devised a simple method for recording intracranial venous pressure in the superior sagittal sinus as it empties into the torcular. The procedure possessed the very distinct advantage of permitting direct observations of the effect of the manipulative procedure upon the pressure of the cerebrospinal fluid, thus affording control of artificial increases in the pressure of the cerebrospinal fluid due to venous obstruction in the cranium. With such technical controls, Weed and Hughson were able to show that in practically every case the pressure of the cerebrospinal fluid was considerably above (5–50 mm.) that of the sagittal sinus. They also presented data which indicated, in accordance with the findings of Dixon and Halliburton and of Becht, that alteration in intracranial venous pressure effected alterations in the pressure of the cerebrospinal fluid in the same direction but of lesser magnitude. Conversely it was shown, in agreement with Dixon and Halliburton, that within the physiological limits tested, alteration in the pressure of the cerebrospinal fluid caused changes of lesser extent but of the same direction in the sagittal venous pressure.

This conception, advanced by Weed and Hughson, that the pressure of the cerebrospinal fluid is practically always above that of the cerebral veins, alone affords basis of explanation for Wegefarth's experiments. Wegefarth (77) demonstrated that an experimental communication between subarachnoid space and superior sagittal sinus remained patent, without hemorrhage into the meningeal cavities, for at least 4 days. Removal of cerebrospinal fluid in these animals, however, resulted in immediate intrameningeal hemorrhage. Such an observation, free from the errors of recording instruments, can be explained only on the basis that normally the pressure of the fluid is above that in the sinus or that the former is constantly being reduced toward the latter.

Analysis of the reliable data concerning these normal relationships seems convincing in demonstrating that the pressure of the cerebrospinal fluid practically always exceeds that of the superior sagittal sinus. In no sense may the two pressures be regarded as identical, for such an identity of pressures is found only in animals in which the technical procedures have resulted in the production of direct communications between sinus and meningeal spaces. In the normal animal intracranial arterial pressure is a factor of importance in the maintenance of the pressure of the cerebrospinal fluid, though slight or slowly effected changes in this arterial tension have but little influence upon the fluid pressure. Thus while dependent upon both intracranial arterial and venous pressures and while influenced passively and in the same direction by both, the pressure of the cerebrospinal fluid may be considered to be relatively independent of both in that normally it maintains an individual, fairly constant level far below that of the intracranial arteries and somewhat above that of the intracranial veins.

It becomes desirable, then, to ascertain the factors which determine the level of the cerebrospinal fluid pressure, but in this inquiry there are practically no data available and the problem becomes speculative. Yet it is instructive to think of the cerebrospinal fluid as being largely elaborated by the cells of the choroid plexuses where the pressure in the blood capillaries is estimated at from 40 to 60 mm. Hg. On the outer side of these cells is the cerebrospinal fluid with its pressure of 110 to 130 mm. of Ringer's solution. After circulating through the cerebral ventricles and subarachnoid space this fluid is largely returned into the venous sinuses of the dura where the pressure (as determined in the superior sagittal sinus) is below that of the cerebrospinal fluid (as determined in the cisterna magna). On such a basis the normal mechanism for the absorption of this characteristic body liquid may well be a simple process of filtration though the factors of osmosis and diffusion are not excluded in the passage of the fluid through the cell-membrane of the arachnoid villus. No determinations of the pressure of the cerebrospinal fluid in the arachnoid villus have been made but it is unlikely that this pressure is markedly different from that of the cisterna magna. Obstruction to any part of the pathway of the fluid results in raising intraventricular tension (cf. Nañagas (53)) thus demonstrating that cerebrospinal fluid can be produced against higher pressures than normally exist in the cerebral ventricles. The normal pressure therefore may be largely determined by the balance between the constant new production within the cerebral ventricles and the absorption into the dural sinuses: this pressure of the cerebrospinal fluid becomes dependent also upon intracranial arterial and venous pressures, not only because of the relation of these latter pressures to the production and absorption of the fluid, but because of the constancy of volume of the intracranial contents.

MODIFICATION OF THE PRESSURE OF THE CEREBROSPINAL FLUID

During the past ten years a number of investigators has studied the alterations in pressure of the cerebrospinal fluid effected by the introduction of various substances into the blood stream or alimentary canal. The subject-matter naturally differentiates itself from the purely mechanical modifications effected by pressure-changes in the cerebral blood vessels. The general findings in this latter group of experiments have been presented in the foregoing section of this review; the pressure-changes effected in the cerebrospinal fluid by solutions of various concentrations and by certain pharmacological agents and tissue extracts will be discussed here.

Solutions of various concentrations. In 1919 Weed and McKibben (75) reported that the pressure of the cerebrospinal fluid could be markedly altered by the intravenous injection of solutions of various concentrations. It was shown that such administration of strongly hypertonic solutions lowered the pressure of this liquid to an extreme degree, frequently producing negative values; with hypotonic solutions (distilled water) a prolonged rise in the pressure of the fluid was obtained. Ringer's solution in large doses produced a temporary increase in the pressure of the cerebrospinal fluid, followed quickly by a return to normal levels. Accompanying these changes in fluid pressure, Weed and McKibben (76) found marked alterations in the volume of the brain, the hypertonic solution producing a small shrunken brain while the hypotonic solution caused an outspoken swelling of the brain-substance. The experimental changes in brain volume were particularly pronounced in animals in which the cranial cavity had been opened by trephining.

These physiological findings have since been abundantly confirmed and clinical applications of the phenomena have been developed. Cushing and Foley (15) demonstrated that similar alterations in the pressure of the cerebrospinal fluid could be brought about by the ingestion of hypertonic and hypotonic solutions. Subsequently Foley and Putnam (31), after verifying the original conclusions, studied similar changes in the pressure of the cerebrospinal fluid which were effected by the intra-intestinal administration of these solutions of various concentrations. And Sachs and Malone (60) reported observations upon the decrease of brain volume caused by the intravenous injection of strongly hypertonic solutions. In addition to these papers, there has appeared a number of reports of clinical application of this experimental modification of fluid pressure or brain bulk—notably those of Cushing and Foley (15), Sachs and Belcher (59), Ebaugh and Stevenson (22), Foley (30) and Hughson (40).

Recently Weed and Hughson (72), (74) have extended the original observations of Weed and McKibben (75); in addition to confirming the initial work in detail, they have presented data showing the general systemic and intracranial vascular alterations effected by these agents. These observations were made over periods of from 2 to 7 hours, under adequate experimental conditions in which the pressures recorded (cerebrospinal fluid, carotid artery, superior sagittal sinus, superficial brachial vein) remained surprisingly constant. The intravenous injection of a large quantity of Ringer's solution was shown to cause a temporary rise in the pressure of the cerebrospinal fluid with increases in both sagittal and brachial venous pressures, the former being the greater. At the end of the injection-period all of these pressures tended to return to their previous levels, normal pressures customarily being attained within 30 minutes. After the intravenous injection of distilled water in similar amount, a prolonged rise in the pressure of the cerebrospinal fluid occurred, accompanied by alterations in both sagittal and brachial pressures. Both of these venous pressures increased during the period of injection and for a few minutes thereafter, the sagittal outstripping the brachial; within 30 minutes these pressures were usually returned to their pre-injection levels, though the pressure of the cerebrospinal fluid was still elevated. With the intravenous injection of strongly hypertonic solutions, the pressure-alterations were most striking, the cerebrospinal fluid, after a frequently occurring rise during the interval of injection, dropping markedly and often exhibiting extreme negative values. The pressure in the superficial brachial vein rose during the period of the hypertonic injection and then rapidly resumed its pre-injection level or a new level slightly below. More significant were the alterations in sagittal venous pressure: here the reaction during the period of injection depended largely on the reaction of the pressure of the cerebrospinal fluid, but following this the sagittal pressure was always lowered to a greater extent than was the brachial venous pressure.

These experiments afforded a unique opportunity for study of the mechanisms which normally control the pressure of the cerebrospinal fluid. Analysis of the data demonstrated that alterations in the pressure of the cerebrospinal fluid could be effected and maintained independently of change in the intracranial and systemic vascular pressures. The most striking similarities in reactions were those between the pressure of the cerebrospinal fluid and that of the brachial vein and sagittal sinus; after the injection of Ringer's solution or of distilled water they exhibited somewhat the same alterations, differing not only in magnitude but in duration. After the intravenous injection of hypertonic solutions, however, the relationships of the pressures were markedly altered, with the pressure of the cerebrospinal fluid profoundly lowered, the sagittal venous considerably and the brachial venous but little if at all. Both brachial and sagittal venous pressures were found to be lower than the pressure of the cerebrospinal fluid in the control-periods; this relationship held after the injection of isotonic and hypotonic solutions but was reversed when hypertonic solutions were given. Arterial changes, when slowly brought about, caused little if any alteration in the pressure of the cerebrospinal fluid, but when abrupt, their influence was marked.

The changes in the pressure of the cerebrospinal fluid, effected by the intravenous injection of solutions of various concentrations, must in the final analysis find their explanation in the alteration of the osmotic pressure of the circulating blood. The injection of a large volume of an isotonic solution was followed by a short-enduring rise of the cerebrospinal fluid pressure which subsided in approximately the same time-interval required for the pressure-changes effected by the hypertonic and hypotonic solutions to reach their maxima. The usual time for maximal reaction of the pressure of the cerebrospinal fluid was noted to be from 25 to 35 minutes after the end of the intravenous injection; in this interval the organism was attempting to compensate for alteration in the volume and salt-content of the blood. With the isotonic solutions, the compensation was one for increased volume of fluid only; this compensation, if judged by the time of return of the cerebrospinal fluid pressure to normal, was rapidly achieved. When, however, not only the volume of the circulating blood was increased but its salt-content relatively diminished as by the intravenous injection of distilled water, two processes of adjustment proceeded. The blood tended to reëstablish its normal salt-content by passage of water into the tissues and possibly into some of the body-fluids, and by attraction of salts from these places; and it also tended to compensate further by alteration of the vascular bed. The increase in the pressure of the cerebrospinal fluid and in the brain volume may be taken to be a rough index of the passage of fluid from blood vessel to tissue; the return of the vascular pressures to normal levels while the pressure of the cerebrospinal fluid remained high, indicated the completion of certain of the phases of readjustment. In the readjustments effected by the organism to the injection of hypertonic solutions, there were somewhat similar phases, yet differing because the great increase of fluid-volume in the circulating blood was not immediate but was due to the attraction of water from the body-tissues and possibly from the body-fluids—a phenomenon shown by the decrease in brain volume and by the reduction of the pressure of the cerebrospinal fluid.

Such an explanation of the phenomena reported leads one naturally to a consideration of the rôle played by the osmotic pressure of the blood in the normal process of absorption of the cerebrospinal fluid. And intimately connected with such a problem is that of the volume of the brain in its relation to the intracranial pressure. At the present time there are available no data which will permit of exact statement; it must be realized that the osmotic changes in the blood, effected by such relatively large injections of solutions of various concentrations, are probably beyond the ordinary physiological limits of change. But if one may judge merely by the anatomical and physiological evidence afforded by subarachnoid injection of isotonic foreign solutions, osmosis and diffusion play subordinate rôles in the normal process of absorption of the cerebrospinal fluid into the blood stream.

Pharmacological agents and tissue extracts. Most of the work done on this subject has been actuated by the tenet that alteration in the pressure of the cerebrospinal fluid, without significant change in intracranial vascular pressures, affords a more reliable means of determining the effect of these various agents upon the rate of production of the fluid than the outflow method. While logically this subject should perhaps be discussed under the heading of the modification of the rate of elaboration of the liquid, it may properly be treated here as an experimental alteration of the fluid pressure.

It may be stated at the outset that there is no unanimity of opinion regarding the effect of either pharmacological agents or tissue extracts upon the pressure of the cerebrospinal fluid, without significant alteration in intracranial arterial or venous pressures. Dixon and Halliburton (20) found that extracts of the choroid plexus, chloroform, ether, urethane, carbon dioxide, amyl nitrite, pilocarpine and other drugs caused a "secretory rise" in cerebrospinal fluid pressure which was independent of the intracranial vascular alterations. Becht (1) has investigated the subject from the same angle, using methods somewhat similar, and has stated that (p. 124) "all the changes in the fluid pressure and in the fluid outflow which have been offered as proof of the secretory mechanism of formation of the cerebrospinal fluids can be traced to alterations in venous and arterial pressures in the skull." Similar conclusions have been reached by Becht and Matill (3) and by Becht and Gunnar (2).

With this conflicting evidence it is of course impossible to do other than reserve opinion in the matter. But certain aspects of the controversy may be commented upon. Dixon and Halliburton and Becht determined cerebral venous pressures in the torcular Herophili—a method which because of the wide divergences in normal pressures reported does not seem adequate though qualitative changes in the pressures are probably fairly accurately shown. While the simple manometric method has been modified by Becht and Gunnar (2), it still has certain limitations in determining a true change in rate of production of cerebrospinal fluid. Of these, the fact that the normal channels of absorption are intact and functioning is the most obvious though Becht has minimized the weight of this objection. There is however a much more formidable disadvantage in that the manometric method cannot take into account the experimental alteration of the volume of the brain. The pressure-changes in the cerebrospinal fluid, effected by the intravenous injection of distilled water, appear to fulfil, after the period of acute vascular change, all of the conditions necessary for the determination that the injection has caused an increased production of fluid—a markedly elevated cerebrospinal fluid pressure with intracranial vascular pressures at the pre-injection levels. The outspoken increase in brain volume under such conditions, however, may well be the sole explanation of the phenomenon; until the experimental variations in brain volume are more fully understood, the evidence obtained by the manometric method should be accepted only with reservations.

MODIFICATION OF RATE OF OUTFLOW OF CEREBROSPINAL FLUID

That certain pharmacological substances may modify the rate of flow of cerebrospinal fluid from a cannula introduced into the subarachnoid space was first determined by Cappelletti (9) in 1900. Employing Cavazzani's (11) method of making a fistula into the cistern region, Cappelletti showed that ether given by intratracheal tube in a curarized dog increased the rate of flow of the fluid from 0.15 to 0.35 grams per 15-minute interval to 4.72 grams for the same interval. A second, a third and a fourth administration gave momentary increases but not of the same extent as the initial. Similar positive results were obtained with pilocarpine, but the increases though obvious were not marked. Very slight augmentation of the rate of outflow was also obtained with amyl nitrite, while atropine and hyoscyamine caused a decrease and on repetition a cessation of the outflow.

Pettit and Girard (55) immediately confirmed and extended these experimental findings of Cappelletti, including in their studies histological examination of the choroid plexuses. And Meek's (49) observations were likewise entirely confirmatory. The scope of the investigation was widened in 1913 by Dixon and Halliburton (19) who studied the effect of a large number of substances upon the rate of outflow of cerebrospinal fluid from an occipito-atlantoid cannula. They were able to classify the substances into four groups according to their effect on this rate of outflow, placing the volatile anesthetics, alcohol, carbon dioxide and extracts of choroid plexus and of brain in the group which caused marked increase in secretion. Slight increases in the outflow were found to be caused by large injections of water or of normal saline, cholesterin, kephalin, atropine, pilocarpine and amyl nitrite. In the large third group of substances which caused no increase or a diminution of secretion were included extracts of the pituitary, of mussel, of pineal and of pia mater, glucose, urea, lecithin, etc., while in the last group where the effect was possibly masked by vascular or respiratory changes, were muscarin, pilocarpine, adrenalin, etc.

Shortly thereafter Dandy and Blackfan (18), obtaining cerebrospinal fluid by introduction of a special cannula through the atlas, found marked accelerations of the rate of output of cerebrospinal fluid following administration of ether and slight augmentations after pilocarpine. With amyl nitrite and extracts of choroid plexus and of posterior lobe of the hypophysis, no change in the rate of output of the fluid was observed.

Realizing the limitations of this technique in that the normal channels of absorption were intact and that the intracranial pressure was reduced to the resistance of the needle, Weed and Cushing (71) in 1915 catheterized the third ventricle and studied the outflow from the catheter whose resistance was established at approximately normal pressure of the fluid. In addition, cerebrospinal fluid was obtained by callosal and occipito-atlantoid punctures with needles of similarly standardized resistances. Under these circumstances the intravenous injection of extract of posterior lobe of the hypophysis was found to increase the outflow of cerebrospinal fluid. This finding was explained by Dixon and Halliburton (21) on the basis that the hypophysial extract had caused a contraction of the bronchioles and consequent asphyxia. Dixon and Halliburton used an intermittent blast for their artificial respiration while Weed and Cushing employed intratracheal insufflation: it seems questionable whether this explanation of the finding will suffice.

At this time also, Frazier and Peet (34) reported that brain-extract increased the secretion of the cerebrospinal fluid as determined by outflow and that thyroid extract decreased it, independently of any vascular changes.

The later studies of the effect of these substances upon the rate of production of cerebrospinal fluid have been made by the manometric method and have been discussed in the preceding section of this review. The limitations of the outflow method were realized by Weed and Cushing (71) in 1915; their modifications introduced control for some of the sources of error but were incomplete. As Becht (1) has pointed out, practically all of this work is of no scientific value because of failures to record simultaneously the intracranial arterial and venous pressures. Using this standard but employing the manometric method, Becht and Matill (3) have concluded that there is no indisputable evidence that the tissue extracts tested have a specific action on the cerebrospinal fluid. And recently Becht and Gunnar (2) reported that adrenalin, pituitrin, pilocarpine and atropine did not increase the production of cerebrospinal fluid, as determined by manometer readings. It is true that the method of recording the rate of outflow of cerebrospinal fluid from a cistern cannula, even with careful determinations of intracranial vascular pressures, yields unreliable data, but in many ways, also, the manometric method fails. Both of these methods, which at the present time are the only technical approaches to the problem, are of questionable value because they both fail to take account of the experimental variation in brain-bulk. When a method which will permit of actual determination of this variable brain bulk, with observations also of cerebrospinal fluid pressure and with a record of intracranial vascular pressures, is devised, data of conclusive value will be obtained. And yet one cannot but lay stress upon the changes in the choroidal epithelium recorded by Pettit and Girard (55) after the injection of pilocarpine. Likewise, as first reported by Cappelletti (9) and since noted by many workers, the rapidly decreasing responses to ether and pilocarpine suggest strongly that the accelerations of flow of cerebrospinal fluid under these conditions were not due solely to vascular alteration, for such ready fatigability would not seem to be associated with a vasomotor reaction.

In this connection it is interesting to speculate upon the possibility of modification of the rate of elaboration of the cerebrospinal fluid, after the intravenous injection of solutions of various concentrations. There is as yet no evidence of value in this regard, though Foley and Putnam presented data which suggested that after the injection of a strongly hypertonic solution, a new ratio between the rate of production and absorption of the cerebrospinal fluid became established. But the final elucidation of this phase of the problem will require additional work before definite conceptions are acquired.

RELATIONSHIP OF CEREBROSPINAL FLUID TO NERVOUS SYSTEM

Many phases of the relationship existing between the central nervous system and the cerebrospinal fluid are of utmost significance in the present discussion. Filling the cerebral ventricles and central canal of the spinal cord, the fluid also completely surrounds the cerebrospinal axis in the subarachnoid space. This double relationship has prompted many observers to look upon the cerebrospinal fluid as constituting a fluid-cushion for the central nervous system within the closed system of cranium and vertebral column. It has also prompted other workers to liken the cerebrospinal fluid to the lymph of the nervous system—a conception which in the light of present knowledge of the lymphatic system is untenable.

Halliburton (37), in a recent lecture, declared that the cerebrospinal fluid serves as the lymph of the brain, though clearly differentiating it from the true lymph of the lymphatic vessels. It seems likely that such a designation, even when correctly qualified as Halliburton has stated it, is apt to introduce error. All modern investigators of the lymphatic system are agreed that true lymphatic vessels do not exist within the dura mater; the older descriptions of such lymphatic vessels were actually descriptions of intradural tissue-channels, subpial tissue-channels, or arachnoidal cell-columns within the dura mater. As Sabin (58) has pointed out, our knowledge of the lymphatic system has advanced so that it becomes now necessary to restrict the term "lymph" to the fluid contained within true lymphatic vessels and not to use it to designate any body-fluid.

But in one respect the cerebrospinal fluid does function as an accessory fluid to the central nervous system. In foregoing sections the drainage of the fluid contained within the perivascular channels toward the subarachnoid space has been commented upon; this fluid really becomes added to the ventricular cerebrospinal fluid in the subarachnoid space. In that sense, then, these perivascular spaces represent accessory drainage channels, uninterrupted by cell-membranes and capable of carrying toward the subarachnoid space the waste products of nerve-cell activity. Lacking a true lymphatic system, the nervous tissue apparently makes use of these perivascular channels as pathways for fluid elimination.

The ultimate connection of these perivascular channels with potential spaces about each nerve-cell indicate the close relationship between the cerebrospinal fluid and the nervous system. And in addition to these rather obvious fluid spaces about the nerve-cells, there is evidence indicating that this fluid-system is intimately connected with the general tissue-channels through the ground-substance of the brain. The general direction of flow of this fluid under normal conditions seems to be toward the subarachnoid space.

But under certain conditions this direction of flow may be reversed so that the cerebrospinal fluid passes from subarachnoid space to nerve-cell. The first of these conditions is that of cerebral anemia in which, as Mott (52) showed by histological study, all of the perivascular, pericapillary and perineuronal spaces are dilated. The author (68) made use of this phenomenon as a means of injecting this perivascular system from the subarachnoid space. The second of these conditions under which the perivascular flow is toward nerve-cell, is brought about by the intravenous injection of strongly hypertonic solutions. This phenomenon was first noted by Weed and McKibben (76) who supplied a foreign solution of sodium ferrocyanide and iron-ammonium citrate to the subarachnoid space at the time when the cerebrospinal fluid pressure was approaching zero, following the intravenous injection of a strongly hypertonic solution. This foreign solution was subsequently found (p. 536) "to have passed from the subarachnoid space along the perivasculars into the substance of the nervous system, reaching the interfibrous spaces in the white matter and the pericellular spaces in the gray." These observations were interpreted as indicating that, under the influence of the intravenous injection of the strongly hypertonic solution, the dislocation of a considerable quantity of cerebrospinal fluid into the nervous system occurred.

Foley (29) has subsequently carried out experiments quite similar to those reported by Weed and McKibben, using the same foreign salts for subarachnoid introduction and intravenous injections of strongly hypertonic solutions. In addition to the findings already detailed, Foley obtained evidence of a retrograde absorption not only by ependyma but by choroid plexuses. The absorption by the ependyma is amply verified by the work of Wislocki and Putnam (79) and Nañagas (53), but these latter workers, using careful histological control, have been unable to obtain evidence of absorption of the foreign salts by the choroid plexuses.

And in work as yet unpublished the writer has repeated many of his earlier experiments done with McKibben, with findings confirmatory in every regard. The intracranial vascular and the cerebrospinal fluid pressures have been determined both before and throughout the period of subarachnoid introduction of the foreign solution, so that definite physiological control is afforded. The results indicate that with the increase of osmotic pressure of the blood, due to the intravenous injection of hypertonic solutions, the cerebrospinal fluid is aspirated into the shrinking nervous system, chiefly along the perivascular channels but also through the ependymal lining of the ventricles. Along these channels, under this extraordinary osmotic pull, actual absorption of the fluid into the vessels of the nervous tissue takes place. The findings suggest a reversal, following the injection of the hypertonic solution, of the normal processes; the osmotic pressure of the blood stream, under these conditions, seems to be a determining factor in the absorption of the cerebrospinal fluid. Interpretation of certain of the experimental observations makes it seem likely that diffusion also plays a part in the process.

RÉSUMÉ

The limitations of this review have made it impossible to more than rather briefly discuss a few of the many problems connected with the cerebrospinal fluid. Many equally absorbing phases have been untouched for one or another reason but an attempt has been made to indicate the type of evidence which has furnished the working hypotheses and to point out the limitations of the procedures on which so many conclusions have been based.

Our present knowledge of the processes of the cerebrospinal fluid in many respects is inadequate. The conception that this characteristic body-fluid is largely produced by the intraventricular choroid plexuses is based not on any single conclusive piece of evidence but on a mass of suggestive data; when considered from all standpoints, however, the hypothesis seems today well established. The current ideas regarding the circulation of the fluid through cerebral ventricles and subarachnoid space are founded largely on exact anatomical evidence, particularly in regard to the structure of the meninges and the use of these intrameningeal channels as fluid-pathways. And likewise, there are firm and reliable data of an anatomical and physiological nature supporting the contention that the cerebrospinal fluid is absorbed largely into the venous system and to a lesser extent into the lymphatic channels. It is possible now to discard the hypothesis of equality between the cerebrospinal fluid pressure and that of the cerebral veins, and to regard the cerebrospinal fluid as being maintained at an individual, relatively independent pressure at fairly constant levels above that of the sagittal venous sinus. The conceptions of pressure-changes effected by the intravenous injection of solutions of various concentrations are substantiated by dependable observations, but it does not seem as yet justifiable to accept, without further control, the data furnished in regard to similar changes brought about by administration of pharmacological agents and tissue extracts. And the same cautions may be urged in regard to the acceptance of conclusions based on the effects of various agents upon the rate of outflow of the fluid.

Yet these problems are but few of the many fascinating subjects of investigation in this field. The interesting questions of the chemical composition of the fluid have not been discussed: is the cerebrospinal fluid a true secretion, a transudate, or a modified dialysate? Likewise, the long-debated problems of the passage of foreign salts, of drugs, etc., from blood stream into the fluid must be left for future review, though with possibly a note of suggestion that these investigations be carried out with control of the cerebrospinal fluid pressure. And so may the many other partially answered questions centering about this fluid be enumerated.

But in this field of research the work of the next few years will solve certain problems; yet the solution of these will but expose wider fields for examination. Here, as in countless other investigations, the study of structure must proceed hand in hand with the study of function, for many of the erroneous conceptions introduced into the literature of the cerebrospinal fluid have been due to failure to give regard to one or other of these basic factors. Future investigations will be the more profitable if the studies be largely along the lines of physiological-anatomical control.

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MILK SECRETION AS RELATED TO DIET[1]

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The following will serve as a rough outline of the various kinds of experiments which have been carried out to throw light on the physiology of milk secretion as related to diet.

1. Experiments on the chemistry of the various food materials and of milk. A review of the chemistry of milk was published by Raudnitz in 1903 (97).

2. Experiments in which the quantity of particular constituents of the food was varied while the composition and yield of the milk were studied.

3. Experiments in which the income and outgo of various nutritional elements and compounds were studied by determining their quantity simultaneously in the food and in the milk and excreta. Such experiments have been used to throw light on a great number of problems; as, for instance, on the digestibility of various compounds, and on the quantities of these required to supply a given milk production, on the manner in which a lactating animal responds to a shortage of some particular compound in the diet, and on such questions as whether milk fat can come from the carbohydrate or protein of the diet.

4. Experiments on the chemistry of the blood of milking animals. For some time past efforts have been made to determine in this way in what chemical form the immediate precursors of the various constituents of milk are carried in the blood. More recently, the chemistry of the precursors of milk having been to some extent determined, efforts have been made to discover how the concentration of these in the blood is regulated, and what effect variations in their concentration have on milk secretion.

5. In a few very recent experiments attempts have been made to determine the relation between milk secretion and the vitamines of the food.

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[1] Published with the permission of the Secretary of Agriculture.

It is of course impossible, in a brief review, to discuss adequately all the experiments that come under the above headings. This article will concern itself chiefly with that part of the field which bears on the problem of the regulation of milk secretion through the diet, taking into consideration particularly the newer work which opens the road toward showing how this regulation is accomplished through changes in the composition of the blood.

I. EXPERIMENTS IN WHICH THE MILK YIELD WAS STUDIED WHILE CHANGES WERE MADE IN THE FOOD. Eckles and Palmer studied the composition and yield of milk in cows which were on generally superabundant rations (24) and on rations generally insufficient to supply their requirements for maintenance and milk yield (25). The results of the experiments with superabundant rations indicated that the milk yield was little or not at all increased by feeding rations more liberal than those required by the accepted standards for feeding dairy cows, and that the composition of the milk was not affected.

The experiments with inadequate rations were carried out partly on cows in the early stage of lactation and partly at a later stage. The cows in the early stage of lactation showed a remarkable power of secreting milk on very inadequate rations. That they used the materials of their own bodies to supply the deficiency was shown by their decreases in body weight. At later stages of lactation the response to inadequate rations consisted more in a reduction of milk yield and less in the mobilization of the body tissues.

The inadequate rations produced no change in the lactose content of the milk.[2] The changes in the concentration of the fat and protein were rather irregular, but the fat content of the milk was increased rather more often than decreased by the inadequate feeding.

Basch reviewed the subject of milk secretion in 1903 (6). He refers to the earlier investigators who studied the effects of changes in particular constituents of the diet on the composition and quantity of the milk yielded, and gives an outline of their results. For citations of work prior to 1903, the reader is referred to Basch's article.

The results of these earlier investigations may be summed up in a few words. There is a general agreement that the quantity of milk yielded is highly dependent on the quantity of protein supplied in the food. It is a very general rule that increased protein in the food results in an increased milk yield, and vice versa. The effects of changes in the quantity of food protein on the composition of the milk are not constant. An increase in the food protein may have no effect on the concentration of milk nitrogen, or may be followed by either an increase or a decrease in this factor. It may similarly be followed by no change or by either of the two possible changes in the concentration of milk fat. But an increase in the quantity of food protein usually results in an increase in the total amount of milk fat secreted.

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[2] Throughout this article the word "content" will be used as above to mean "concentration," not the total amount of the constituent in question secreted.

The results obtained by changing the quantity of fat in the ration are even more variable than those in regard to protein. Some authors find that an increase of fat in the food is followed both by an increased milk yield and by an increased concentration of milk fat; others get neither of these results; still others get one without the other.

Comparatively little work has been done on the effect of changes in the carbohydrate of the food. It has been found, however, that where moderate amounts of carbohydrates are added to or subtracted from the ration, little or no change follows in either the composition or quantity of the milk.

The results which have just been summed up must be considered in relation to the possibility of the conversion of one food constituent into another. Milk protein must, of course, come ultimately from the protein of the food. But many investigators, whose work has been reviewed by Lusk (76, pp. 223–247) have shown that food protein may be converted into either fat or carbohydrate in the animal body; and Jordan and his collaborators (67), (68) have shown specifically that the carbohydrate of the food may be converted into milk fat.

It is easy to see, therefore, why the protein of the food should occupy a dominant position in regulating milk yield. If the food protein is insufficient for the quantity of protein that is being excreted in the milk, the animal must either take protein from her own body or reduce the secretion of milk protein; it is not surprising that she usually does both at the same time. In the cases of fat and carbohydrate, on the other hand, there is a much larger number of possibilities.

It is not surprising, in view of these considerations, that the results of changes in diet on the composition of milk have been rather variable. The effect on milk yield of any change in diet will depend on a great number of accessory experimental aspects—on the amount of the change, on the level at which the change is made, on the quantity of other constituents being fed, on the nutritive state of the animal, and on other factors which the reader can easily imagine. The more modern investigations have kept these factors more or less in view.

Shortly after the publication of Basch's review, Morgen and a number of collaborators attacked the problem of the effect of food on milk yield (89), (90), (91), (92). These authors carried out an extensive set of investigations on the effects of diet on the composition and yield of milk, using sheep and goats as their experimental animals. Their results may be summarized as follows:

When the fat in the ration of a milking animal amounts to less than 0.5 gram daily per kilogram of animal, an addition of fat to the ration, as a substitute for carbohydrate, has a marked effect in increasing the percentage of fat in the milk, as well as the total milk yield. The milk yield and the fat content of the milk continue to increase with the addition of fat to the ration up to about 1 gram per kilogram of animal. The further substitution of fat for carbohydrate has no effect on milk yield, except in unusual cases. When the milk yield is increased by the addition of fat to the ration as described above, the nitrogen content is generally reduced.

Additions of protein to the ration at almost any level tend to increase the milk yield. The nitrogen content of the milk is sometimes increased and sometimes decreased; the fat content is generally lowered, but the total amount of fat secreted is generally increased.

The authors did not try in any of their experiments to use a basal ration in which the carbohydrate was reduced to a minimum. They did, however, try adding moderate quantities of carbohydrate to rations which already contained the usual proportion of that material, and found that it had little or no effect on either the composition or the yield of milk.

The concentration of lactose in the milk was not significantly affected by any of the changes in ration which they carried out.

In recent experiments Cary has studied the effects on milk yield of making large reductions in either the protein or the carbohydrate of the food, starting from an approximately normal ration (15). A large reduction in either of these constituents of the food is followed by a marked falling off in milk yield which begins a few hours after the food is changed. A shortage of protein in the food reduced the nitrogen and fat contents of the milk, the sugar content remaining unchanged. A marked shortage of carbohydrate in the food also reduced the nitrogen content of the milk, but in this case the fat and sugar contents remained unchanged.

Fingerling (33) has carried out experiments on goats in which the quantities of calcium and phosphorus in the rations were varied while the yield and composition of the milk were studied. Reduction in the calcium and phosphorus of the rations at first produced no effect on the milk yield; after several weeks the yield of milk began to fall off progressively while the concentrations of calcium and phosphorus in the milk rose; these changes were largely reversed by restoring calcium and phosphorus to the rations.

Jordan, Hart and Patten (69) carried out two experiments in which the phosphorus content of the ration was varied while the yield and composition of milk were studied. The phosphorus concentration in the milk was not affected, but the percentage of milk fat was noticeably reduced on the low phosphorus ration, and vice versa. The milk yield was little changed by the change in the food phosphorus; it tended to be a little higher on the low than on the high phosphorus ration.

The experiments of Fingerling and of Jordan, Hart and Patten show that milk yield is not affected by changes in the calcium and phosphorus content of the diet until after a considerable interval. Recent experiments of Meigs and Woodward (84a) indicate that the full effects on milk yield of dietary deficiencies in one or both of these elements may not be exhibited for several years, and that the yield of any given lactation period may depend to a considerable extent on whether or not the lactating animal received sufficient calcium and phosphorus in the rations preceding the beginning of that period.

The results which have just been given may be summarized as follows:

Both the milk yield and the composition of the milk may be considerably influenced by changes in the ration. Of the three main organic constituents of the milk, the carbohydrate is by far the most constant; its concentration is not affected by any of the changes in ration so far studied. Reductions in the protein of the ration result very generally in reductions in the milk yield. The nitrogen content is usually either decreased or unaltered, and the fat content may be altered in either direction or may remain unchanged. Reductions in the fat of the ration have little or no effect on the composition and yield of milk until the amount of fat fed falls below 1 gram per kilogram of animal. When it falls below this level, the milk yield and the fat content of the milk are generally reduced, while the nitrogen content is increased. Small changes in the amount of carbohydrate fed, comparable in absolute magnitude with those which have been studied in the cases of protein and fat have no immediate significant effect on either the yield or the composition of the milk. Large reductions, amounting to 50 per cent of the total nutrients contained in the ration, reduce the milk yield and the concentration of nitrogen in the milk, while leaving the fat and carbohydrate content practically unchanged. The effects of changes in the amounts of carbohydrate fed when the total quantity of this constituent in the basal ration is reduced to a minimum, have not been studied.

A reduction in the amount of phosphorus in the ration may have the effect of reducing the fat content of the milk, while leaving the phosphorus content and the total milk yield practically unchanged. Simultaneous marked reduction of the calcium and phosphorus in the ration results after an interval in a reduction in the milk yield. The calcium and phosphorus contents of the milk are actually increased, though the total amounts secreted are reduced. The full effects on the milk yield of a shortage of calcium or phosphorus in the ration may not show themselves for a very long time.

The above results are, in many cases, not such as would be expected from a cursory consideration of the subject. In some cases they suggest hypotheses regarding the nature of the relation between food supply and milk secretion; in others they hint at metabolic relations which demand careful consideration.

It may first be pointed out that there is a rather sharp contrast between the effects of changing the organic constituents of the ration and those of changing the inorganic constituents. If either the protein or the total nutrients of the ration be sharply reduced, the milk yield generally begins to fall off within a few hours. In Fingerling's experiments, on the other hand (33), although the relative reduction in the amount of calcium and phosphorus fed was larger than the reductions in the organic constituents studied by the other authors, nevertheless the milk yield was not much affected for several weeks. Fingerling followed the calcium and phosphorus balances in his experiments, and found that his animals began to lose these elements from their bodies, as soon as they were put on the low mineral rations. His results give an interesting picture of the quantitative relations for such phenomena, the rates at which calcium and phosphorus are lost from the bodies of milking animals on deficient rations, the time at which milk yield begins to be affected, the manner in which it is affected, and the extent to which it may be restored by a return to adequate rations.

There are numerous other experiments which show that milking animals may remain for considerable periods in marked negative calcium and phosphorus balance without showing much drop in milk yield.

Among these those of Hart and of Forbes and their collaborators are particularly to be mentioned (52), (42), (43), (44).

The milk yield is much more immediately affected, therefore, by a serious shortage of the organic constituents of the ration than by a shortage of calcium and phosphorus. But the reader must not take away the impression that the milking animal adjusts herself to a shortage of protein, fat or carbohydrate merely by reducing the quantity of the missing material secreted in the milk. The work of Hart and his collaborators (53), (54), (55), (56), (57) shows that cows on an inadequate protein ration go into negative nitrogen balance and fall off in their milk yield at the same time. The work of Jordan and his collaborators, on the other hand (67), (68), shows that cows on an inadequate fat ration may respond by manufacturing fat from carbohydrate as well as by reducing the quantity of milk fat secreted. And there is no doubt that the body stores of fat and carbohydrate are frequently called on when the necessity arises. The situation may be summed up by saying that a serious shortage in any of the food constituents so far studied either throws the milking animal into immediate negative balance as far as that constituent is concerned, or causes her to begin manufacturing the constituent from some other material. In the cases of protein, fat and carbohydrate, however, a serious shortage also has the effect of immediately reducing the amount of milk secreted, whereas a serious shortage of calcium and phosphorus may not affect milk secretion for a considerable time.

The work considered so far indicates that protein plays a predominant part in regulating milk secretion. The authors quoted above frequently make the statement that protein "stimulates milk secretion," and the truth of the statement is borne out by the following aspects of the results. In the first place it is shown by the work of Morgen and his collaborators (90), (91), (92) that changes in the amount of protein fed are likely to affect the milk yield at almost any level of protein feeding, and that the changes in total milk yield produced are large in comparison to those produced by comparable changes in the quantity of either fat or carbohydrate fed. In the second place it comes out in all the pertinent work, but particularly again in that of Morgen and his collaborators, that changes in the quantity of protein fed have a marked effect on the whole milk yield, with subordinate effects on the composition of the milk. Changes in the amount of fat fed, on the other hand, if they have any effect at all, produce comparatively large changes in the fat content of the milk and only moderate changes in the total milk yield.

It is true that large changes in the amount of carbohydrate fed (which mean also large changes in the total energy of the ration) have marked effects on milk yield. But Cary (15) has brought out the suggestive point that a reduction in the carbohydrate of the ration causes a reduction in the nitrogen content of the milk. The significance of this will be much clearer after it has been possible to take up another phase of the subject.

There are other points besides this in the investigations so far considered which call for further research. Why, for instance, should the calcium and phosphorus content of the milk be actually increased when the food content of the same elements is diminished? Why should the amount of phosphorus in the ration have an effect on the secretion of milk fat? Finally, how do changes in the quantity of this or that constituent of the food act on the mammary gland to regulate milk secretion?

The reader will hardly hope for complete and satisfactory answers to these questions. But the most promising method of approach is obviously to go deeper into the chemical changes undergone by the food materials within the body of the milking animal. A number of investigators have thrown light on this field by determining the precursors of the milk constituents in the blood, and the work along this line will next be taken up.

II. THE PRECURSORS OF THE MILK CONSTITUENTS IN THE BLOOD.

1. The precursor of lactose. In 1884 Bert reported experiments (7) which bear on the problem of the precursor of milk sugar. He removed the mammary glands of goats; then had the animals bred and allowed them to give birth to their young. He examined the urine of the mothers just after the young were born and found that it contained sugar while the urine of normal goats did not contain sugar just after the birth of the young. Bert concludes that sugar is thrown into the blood from some source—probably the liver—just after parturition, in order to supply the sugar to be secreted in the milk. When the mammary gland is not present to dispose of the extra sugar, the latter escapes in the urine.

In 1909 Porcher published the results of investigations (96) which confirmed and extended the work of Bert. It was shown that the post-partum glycosuria of females deprived of their mammary glands was accompanied by hyperglycemia; that the sugar in question was glucose and not lactose; and that the post-partum glycosuria persisted for only a short time.

Results rather opposed to those of Bert and Porcher have been reported by Moore and Parker (88), by Marshall and Kirkness (83), and by Foà (34), and have given rise to more or less controversial literature. It seems worth while to give a short discussion of this controversy, as the results of Bert and Porcher lend support to two important propositions in regard to the physiology of milk secretion.

The two propositions are, first, that during lactation dextrose is actively thrown into the blood by some organ, probably the liver; and second, that dextrose is, therefore, probably the precursor of milk sugar. The facts, as observed by Bert and Porcher, are that in goats from which the mammary glands have been removed parturition is followed for a few hours by hyperglycemia and glycosuria. Porcher also found that hyperglycemia and glycosuria followed, for a few hours, the operative removal of the mammary glands from lactating goats. In both kinds of experiments the hyperglycemia and glycosuria remain at their height for less than twenty-four hours and disappear entirely within three or four days. Both Bert and Porcher failed to get these results in guinea pigs, or got them to only a slight extent.

The increased blood sugar and urinary sugar are, therefore, according to the results of these authors themselves, to be regarded as largely in the nature of a significant accident. In goats something happens very soon which either inhibits or disposes of the surplus sugar supply, so that it does not appear in the urine. In guinea pigs, for some reason, the increased blood sugar and urinary sugar do not appear at all.

The results of Marshall and Kirkness, Moore and Parker, and Foà do not really contradict this point of view. Marshall and Kirkness worked only on guinea pigs and obtained the same negative results as Bert and Porcher. Moore and Parker worked on goats, but they are vague about the exact time after parturition at which they examined the urine. In one of the two goats experimented on, the urine did show a small increase in reducing power after parturition. Foà reports only two experiments; in these the mammary glands were removed from lactating goats. The operations each lasted seven hours, and the urine was not examined until three hours after they were completed. As anesthesia and operations are themselves likely to have an effect on blood sugar (100) this form of experiment is less satisfactory than that in which the mammary gland is removed in a preliminary operation and the blood and urine examined after parturition—particularly where the operative procedure takes so long a time as in the experiments just reported.

A more direct method of determining the precursor of lactose in the blood has been devised by Kaufmann and Magne (70). These authors took samples of blood from the jugular vein and from the abdominal subcutaneous vein of cows, approximately simultaneously. The abdominal subcutaneous or mammary vein carries blood coming from the udder (102, pp. 609, 721), whereas blood from the jugular vein may be regarded as equivalent to the blood of the mammary artery, as far as the materials involved in milk secretion are concerned. In milking cows, therefore, it is to be expected that the blood from the mammary vein should contain less of the precursors of the various milk constituents than blood from the jugular vein. Kaufmann and Magne found that in milking cows the mammary blood contained, on the average, about 18 per cent less sugar than the jugular blood: in different experiments the figures varied from 7 to 30 per cent. Kaufmann and Magne controlled these results by repeating the experiment on a dry cow, and found that in this case the samples of blood obtained from the two different sources above mentioned contained equal amounts of sugar. The results of this investigation give strong support to the view that the dextrose of blood is the precursor of milk sugar.

Foà (34), (35) has used still another method to determine the precursors of the various constituents of milk. He removed the mammary glands of sheep, kept them in a vessel full of Ringer's solution at body temperature, and perfused them with Ringer's solution, with various mixtures of blood and Ringer's solution, and with Ringer's solution to which various substances had been added. He determined the quantity of dextrose in the various kinds of perfusion mixtures both before and after they had been caused to circulate through the gland, and determined also the nature of the fluid secreted by the gland when various perfusion mixtures were caused to circulate through it. He found that when a mixture of blood and Ringer's solution was caused to circulate through the gland, milk containing lactose was secreted. At the same time the dextrose content of the perfusion mixture was shown to decrease. The concentration of lactose in the milk secreted could be increased by adding dextrose to the perfusion mixture. When Ringer's solution with dextrose added was used as the perfusion mixture, the gland secreted a watery fluid containing lactose. No lactose was obtained in the fluid secreted by the gland when Ringer's solution alone was used as the perfusion fluid, or with Ringer's solution with galactose added to it. Foà's results taken all together furnish strong evidence for the view that the dextrose of the blood is the precursor of lactose.

My colleague, Mr. C. A. Cary, has thought it worth while to repeat the experiment of Kaufmann and Magne, and has kindly given me permission to report his unpublished results here. Their article on the subject is exceedingly brief, and there is a possibility that if they did not take their samples of mammary and jugular blood strictly simultaneously, their results might have been disturbed by the tendency of the blood sugar to vary as the result of pain, anxiety, etc. (13, pp. 66–80), (100). Mr. Cary has collected samples of jugular and mammary blood from milking cows strictly simultaneously. A cannula is first inserted in the mammary vein and left stoppered until after the jugular cannula has been inserted. Blood is then collected from the two cannulae at the same time. It was found that the mammary blood contained about 24 per cent less sugar than the jugular blood, and the mammary plasma, about 32 per cent less than the jugular plasma.

There is, then, a large mass of evidence showing that lactose is derived from the blood sugar, and there is no contradictory evidence. The controversy between Bert and Porcher, on the one hand, and Moore and Parker, Marshall and Kirkness, and Foà, on the other, may be disregarded as far as this question is concerned. Moore and Parker, Marshall and Kirkness, and Foà claim only that the concentration of blood sugar is not increased after the removal of the mammary gland from milking animals; and there is no necessary connection between this contention and the question whether or not lactose is normally derived from blood sugar.

2. The precursor of milk fat in the blood. Foà used his method of experimentation to throw light on the origin of milk fat. He found that when Ringer's solution alone was perfused through the excised mammary gland, a watery solution was excreted which contained no fat (35). But when he perfused Ringer's solution in which olive oil or tri-olein had been emulsified, the gland secreted a watery solution containing fat. The fat secreted was in globules which had the microscopic appearance of globules of milk fat, and it had a lower iodine number than the fat of the perfusion fluid. Foà concludes that milk fat is derived from the triglycerides of the blood.

The author, in conjunction with others, has used the experimental method of Kaufmann and Magne to throw light on the question of the origin of milk fat; but, in order to discuss these experiments, it will be necessary to consider the nature of the phosphorus compounds contained in blood. It is now well established that the great majority of all the phosphorus contained in normal blood plasma is divided between two classes of compounds—the phosphatids and the inorganic phosphates (1), (2), (46), (47), (48), (99). Our own results indicate that there is no phosphorus of any kind in plasma except these two (84). Bloor (11) finds that a third kind of phosphorus makes up about 4 per cent of the total; and Feigl (27), (28), (29), (30), (31) finds about 6 per cent.

Our experiments above referred to (84) were primarily designed to determine whether the phosphorus of milk was derived from the phosphatid or from the inorganic phosphate of the blood plasma. Samples of blood were collected approximately simultaneously from the jugular and mammary veins of milking cows; the plasma was separated from the corpuscles and analyzed for phosphatid and for inorganic phosphate. When the experiments were carried out without too much disturbance to the animals used as subjects, it was found that the mammary plasma contained less phosphatid than the jugular and more inorganic phosphate. The changes in the two kinds of phosphorus nearly offset each other so that the jugular and mammary plasma contained nearly equal total quantities of phosphorus. When the animals were much disturbed by the experimental procedure, the jugular and mammary plasma contained equal quantities of phosphatid, but the mammary plasma contained more inorganic phosphate, and, therefore, more total phosphorus than the jugular. These results have been interpreted to mean that milk phosphorus and milk fat are derived from the phosphatid of the blood plasma. The phosphatid of the plasma probably contains about one part by weight of phosphorus to 20 parts by weight of fatty acids, while milk contains about one part by weight of phosphorus to 50 parts of fat. If, therefore, the mammary gland takes from the plasma enough phosphatid to supply a given quantity of milk with fat, it gets with it more than twice as much phosphorus as is required for the same quantity of milk. The surplus phosphorus must be returned to the blood, and this is taken to be the explanation of the fact that the mammary plasma regularly contains more inorganic phosphate than the jugular. Disturbance of the cow tends to stop the taking up of phosphatid by the mammary gland, but not the backflow of inorganic phosphate from the gland to the blood. For this reason, the cows which have been much disturbed before and during the collection of the blood samples show an increased quantity of inorganic phosphate in the mammary plasma and no change in the phosphatid content. The experimental results described above will be further considered later, and their relation to the results of Foà on fat secretion and to certain results of Bloor will be discussed.

3. The precursor of milk protein in the blood. Foà (35) tried some experiments on the excised mammary gland of the sheep to throw light on the derivation of milk protein, but his results were negative. He perfused the gland with Ringer's solution to which various forms of protein had been added, but never found any protein in the fluid secreted under such circumstances. He concludes that milk protein is derived from some still unknown material in the blood.

The recent epoch-making work on protein metabolism of Delaunay (20), (21), (22), Folin (36), (37), (38), (39), (40), (41), Van Slyke (103), (104) and their followers suggests that milk protein may be derived from the free amino-acids of the blood. Cary has recently employed the experimental method of Kaufmann and Magne to determine this point (14). He collected samples of jugular and mammary blood approximately simultaneously from both milking and dry cows. The amino-acid nitrogen of the mammary blood was markedly lower than that of the jugular blood in the milking cows, whereas the two kinds of blood contained the same concentration of amino-acid nitrogen in the dry cows. The results form very complete and satisfactory evidence for the view that the milk proteins are derived from the free amino-acids of the blood.

4. General discussion of the precursors in the blood of the various constituents of milk. Evidence has been adduced to show that lactose is derived from the dextrose of the blood, that milk fat is derived from phosphatid, and that milk protein is derived from free amino-acids. It seems worth while to point out, in the first place, that each of the three propositions stated above gains strength from the other two, when the experimental results on which all three are based are compared quantitatively and in detail; and, in the second place, that the three propositions may be taken together as indicating a sort of hypothesis of secretion which is *a priori* probable, and which suggests an explanation of certain aspects of the subject sometimes regarded in the past as rather mysterious.

Cow's milk is a fluid with a fairly constant composition. Typical milk may be considered to contain 3 per cent protein, 4 per cent fat and 5 per cent lactose. If it may be assumed that in cows giving the same quantities of milk the rate of blood flow through the mammary gland is approximately the same, then the amounts by which the precursors of the three above-named constituents of milk are reduced in the mammary blood ought to bear a more or less fixed relation to each other in the case of cows giving approximately the same quantitites of milk.

In the experiments of Kaufmann and Magne, in those of Meigs, Blatherwick and Cary, and in those of Cary, the cows were usually giving about 10 liters of milk daily. Kaufmann and Magne found the sugar in the mammary blood of their milking cows about 15 mgm. per 100 cc. lower than that in the jugular blood. In the unpublished experiments of Cary above referred to, the sugar was determined in mammary and jugular plasma as well as in mammary and jugular blood; and it was found that the sugar taken from the blood by the mammary gland came entirely from the plasma, the sugar content of the corpuscles remaining unchanged during their passage through the mammary gland. The reduction of 15 mgm. per 100 cc. blood found by Kaufmann and Magne would be equivalent, therefore, to a reduction of 22 mgm. per 100 cc. plasma. Cary found a reduction of 20 mgm. per 100 cc. in the mammary plasma.

In the two successful experiments on the phosphatid content of the jugular and mammary plasma by Meigs, Blatherwick and Cary, the phosphatid phosphorus was found to be lower by 0.74 and 0.60 mgm. per 100 cc. respectively in the mammary plasma. This would represent reductions amounting to 14.8 and 12 mgm. of phosphatid in the mammary plasma.

In Cary's experiments on the amino-acid nitrogen of jugular and mammary plasma in milking cows, the amino-nitrogen was found reduced by from 0.39 to 0.97 mgm. per 100 cc. in the mammary plasma, the average reduction being 0.69 mgm. As the alpha amino-nitrogen of the milk proteins constitutes about 70 per cent of their total nitrogen, this would represent a reduction of about 1 mgm. total milk-protein nitrogen per 100 cc. plasma, which would be equivalent to about 6 mgm. of protein.

On the supposition that the milk of the cows used in these experiments contained 5 per cent lactose, 4 per cent fat and 3 per cent protein, the precursors of these three constituents should have been taken out of the plasma passing through the mammary gland in the following proportions. For every 20 mgm. of sugar taken from the plasma there should have been taken phosphorus equivalent to 16 mgm. of fat and amino-nitrogen equivalent to 12 mgm. protein. The figures actually found and given above are 15 to 20 mgm. sugar, phosphorus equivalent to from 12 to 15 mgm. fat, and nitrogen equivalent to from 3 to 9 mgm. protein. The figures for sugar and fat secretion bear very nearly the expected relation to one another, while the figures representing the secretion of milk protein are somewhat lower than would be expected.

But when it is considered that the concentrations of sugar, phosphatid and free amino-acids in blood plasma are all quite low, and that small errors in estimating the actual amounts of these materials present in a given sample would be represented as much larger errors in comparing the differences between jugular and mammary plasma, it seems remarkable that the results actually found should be as close as they are to those that would be expected. Taken together, they furnish strong evidence for the view that the lactose, fat and protein of the milk are respectively derived entirely from the dextrose, phosphatid and free amino-acids of the blood.

The three sets of observations above discussed suggest a working hypothesis of secretion which is *a priori* probable. The mammary gland is called upon to secrete large quantities of protein, fat and carbohydrate at a rapid rate. It is hardly probable, therefore, that it manufactures its products from widely different chemical compounds brought to it by the blood. But it has been clear for some time that casein and lactose, and probably milk fat also, are specific products of the mammary gland. The two former are certainly not present in the blood under ordinary circumstances. Even if the constituents of milk could be demonstrated in the blood it would be difficult to see how the mammary gland could abstract them for the purpose of making milk. Casein and fat are highly indiffusible substances, and milk contains sugar and fat in much higher concentration than does blood. It is very difficult to conceive a mechanism by which such materials could be made to diffuse rapidly from a region of lower concentration to one of higher concentration. But the observations given offer some escape from all these difficulties. To suppose that the mammary gland converts dextrose into lactose, phosphatid into fat, and free amino-acids into casein, leaves undoubtedly many chemical problems still to be solved. But it is conceivable that such changes could take place at a rapid rate and without any great expenditure of energy. Further, dextrose, phosphatid and free amino-acids are more diffusible substances than lactose, triglycerides and proteins; to suppose that the constituents of milk pass the borders of the mammary cells in the form of the three former substances, and are converted, immediately on entering the cells, into the less diffusible latter three compounds, furnishes a rough explanation of the mechanism of secretion, which does not contradict any of the laws of energetics.

The dextrose, the phosphatid and the free amino-acids of the blood plasma are spoken of in this article as the "precursors of the milk constituents." But this is really a one-sided view of them, with no further basis than that milk secretion is the subject of the article. There is little doubt that the three materials above-mentioned are really the general currency of metabolism—the chemical forms in which proteins, fat, and carbohydrate are distributed by the blood to all the organs and tissues of the body.

5. The relation between blood flow through the mammary gland and milk secretion. Cary (14) found no relation between the rapidity of milk secretion and the degree of difference in amino-acid nitrogen concentration as between the jugular and mammary blood plasma. Most of his experiments were carried out on cows giving about 10 liters of milk daily; and while there was considerable variation in the degree of difference between jugular and mammary plasma in these experiments, there was no tendency for the differences to be greatest in the cows which were giving the most milk. One experiment was carried out on a cow which was giving more than twice as much milk as any of the others, and in this case the difference in the amino-nitrogen content of the jugular and mammary plasma was practically the same as the average for the other experiments.

This aspect of his results suggests that the milk yield is in general nearly proportional to the rate of blood flow through the udder. The proposition is probable from general considerations and is supported by a good deal of independent evidence. That given by Roehrig (98) is impressive; and it is well known to dairymen and now statistically demonstrated that there is a fairly close correlation between the size of the milk veins and productiveness in cattle (45).

6. The effects of pain, anxiety, etc., on milk yield. The experiments of Meigs, Blatherwick and Cary on fat secretion indicate that this process is stopped by an even slight disturbance of the cow's comfort. Cary's experiments on protein secretion, on the other hand, do not show any such relation with regard to this latter process. With regard to sugar secretion, evidence on the point at issue is difficult to obtain, because pain and anxiety have a tendency to change the sugar concentration in the whole body of blood (13, pp. 66–80) (100). But, as far as they go, the experiments on record point to the view that the secretion of milk sugar is not noticeably interfered with by pain and anxiety.

There is some independent evidence indicating that fat secretion is more interfered with by pain and anxiety than the secretion of the other constituents of milk. It is commonly thought by dairymen that the percentage of fat in milk is likely to be reduced when cows are chased about by dogs or otherwise disturbed, and when they are in heat. I have observed this tendency occasionally, though I have not carried out any special research on the subject.

7. The opposition between Foà's conclusions and those of Meigs, Blatherwick and Cary. The results of Meigs, Blatherwick and Cary indicate that milk fat is derived entirely from the phosphatid of the blood plasma. The disappearance of phosphatid from the plasma which occurs during its passage through the mammary gland is sufficient to account for all the milk fat secreted. Further, the very considerable backflow of inorganic phosphate from the gland to the blood would be difficult to account for if it were supposed that any considerable part of the milk fat were derived from non-phosphorized fat in the blood.

The work of Foà, on the other hand, indicates that milk fat may be derived from triglycerides in the blood. Foà found that a fluid containing fat was secreted by the excised mammary gland when it was perfused with Ringer's solution to which olive oil or triolein had been added. That the olive oil and triolein had not simply filtered through the gland cells into the ducts is indicated by the facts that the fat in the fluid obtained from the duct was in the form of globules with the microscopic appearance of those normally seen in milk, and that it had a lower iodine number than the fats mixed with the perfusion fluid. That the fat in the fluid issuing from the duct was not simply fat which had been present in the gland cells before the beginning of the experiment and had been washed out into the ducts by the perfusion fluid is shown by the fact that when Ringer's solution without fat was perfused through the vessels, there issued from the duct a fluid which contained no fat.

The chief objection to Foà's experiments is that, as he himself admits, the gland became edematous quite early in the procedure, and that the collection of the fluid issuing from the duct, which was afterwards analyzed, was continued after the edema had been noted. It seems quite likely that the edematous gland cells would give passage to substances which would not pass through the normal mammary cells. It is true that the fat in the fluid issuing from the duct in Foà's experiments had a different iodine number from that in the perfusion fluid; but it is not unlikely that the bodies of the mammary cells might retain some portion of their capacity to alter the character of fats, even after the cell surfaces had become permeable to materials which would not ordinarily go through them.

III. THE CONTROL OF MILK SECRETION THROUGH THE CONCENTRATIONS OF AMINO ACIDS, DEXTROSE AND PHOSPHATID IN THE BLOOD. The investigations reviewed in section I show that changes in diet often produce marked changes in milk secretion, and they bring up the question how milk secretion is controlled through the diet. There can be little doubt that diet exercised its influence by producing changes in the composition of the blood. What is the nature of these changes?

The hypothesis that occurs first to most minds is that a shortage of any particular dietary constituent will bring about a reduction in the concentration of the corresponding milk precursor in the blood. It is natural to suppose that the result of this change will be a reduction in the concentration of the corresponding constituent of the milk, and perhaps also a reduction in the total amount of milk secreted.

The experiments in which the composition and yield of milk have been followed while changes were made in the diet furnish evidence for thinking that the above outlined hypothesis is correct in the case of fat. Reduction in the dietary fat below a certain level produces a marked decrease in the concentration of milk fat and, at the same time, a considerable reduction in the total quantity of milk secreted (89), (90), (91), (92). But in no other case is the evidence so clear. Changes in the quantity of dietary protein produce changes in the quantity of milk secreted through a wide range in the level of protein feeding (90), (91), (92), but the accompanying changes in the concentration of milk protein are irregular. The lactose content of milk is not noticeably influenced by any of the dietary changes so far studied. And the concentrations of calcium and phosphorus in milk may be actually increased by feeding a ration short in these elements (33).

Further light on the question of the control of milk secretion through the diet must be sought in a study of the manner in which the concentrations of the several milk precursors in the blood are affected by various circumstances. The most satisfactory way of studying the question is to follow simultaneously the concentrations of the milk precursors in the blood and the composition and yield of milk while appropriate changes are made in the diet. But few such experiments have been carried out up to the present time; and, while waiting for their number to be increased, it is pertinent to consider the considerable quantity of work already at hand which bears on the general question of the factors which influence the concentration of amino-nitrogen, glucose, phosphatid and calcium in the blood.

Blood is a complicated mixture containing many unstable chemical compounds. It is not surprising, therefore, that the literature contains contradictory figures for the concentration of practically every blood constituent which has been studied. In estimating the value of these results, the following considerations must be kept in mind.

If the concentration of a given constituent of the blood is really constant, it is easy to see how inadequate chemical methods for its determination might make it appear variable. But if the concentration of a given constituent is really variable, it is extremely difficult to see how inadequate chemical methods could make it appear constant. Other things being equal, therefore, the work of those investigators who obtain constant series of results is to be accepted in preference to that of those who obtain more variable series.

In cases where several independent investigators have obtained constant series of results all agreeing closely with one another, the case becomes stronger; and it is justifiable to summarily dismiss non-concordant results obtained by other investigators, unless these latter can show why their methods should be preferred to those of their opponents.

This course will be adopted in the following discussion. In such a review as this it is impossible to give either a critical discussion of the very complex methods of blood analysis or a detailed account of the great mass of wild results which have been obtained in the field. Only those figures will be considered, therefore, which, in the light of our present knowledge, appear to be fairly reasonable.

1. Relation between milk secretion and the free amino-acids of the blood. An admirable review of the modern work on protein metabolism has recently been published by Van Slyke (105). The experimental results show that the food protein is converted in the intestinal tract to free amino-acids, in which form it enters the blood. The blood from the intestinal tract passes first to the liver, and in that organ a considerable proportion of the amino-acids resulting from protein digestion is deaminized. The remainder goes on into the general circulation, serving, for some time, to increase the concentration of amino-nitrogen in the systemic blood, but being gradually absorbed by the tissues and either built into new protein or deaminized and oxidized.

Still more recently Cathcart has published a new edition of his monograph on the physiology of protein metabolism (16), which contains an extensive bibliography.

In section I work was reviewed which indicates that milk yield is highly dependent on the protein supplied in the food; section II contains work which shows that milk protein is derived from the free amino-acids of the blood. It seems natural, in view of these facts, to suppose that milk yield is largely regulated by changes in the amino-acid mixture circulating in the blood.

An obvious method of investigating this problem is to follow the milk yield and the amino-nitrogen content of the blood, while changes are made in the diet. But such experiments cannot be intelligently planned without taking into consideration the various aspects of metabolism which are likely to influence the results.

The blood carrying the products of digestion from the intestinal tract passes first to the liver, and the results of Van Slyke (105) indicate that that organ immediately begins to deaminize some of the amino-acids which it receives. But we know very little in regard to the details of this hepatic activity. As the amino-nitrogen content of the blood is so small, and as so large a proportion of it is removed by the mammary gland in milking animals (14), it is natural to suppose that the liver's deaminizing activity is more or less selective—that it improves the quality of the amino-acid mixture in the general circulation by deaminizing chiefly those compounds which are present in the food in larger proportion than is required for milk secretion, tissue growth or other metabolic activities. But this point of view is by no means strictly proven; and, even if it were, it would still be possible that a marked change in the quality of the protein supplied in the food might produce a less marked but still important change in the quality of the amino-acid mixture circulating in the blood.

The quality of the amino-acid mixture in the blood might be influenced in another way, which is particularly likely to occur in milking animals; namely, by a change from a positive to a negative nitrogen balance. In the case of an animal in positive nitrogen balance, the amino-acids which enter the general circulation pass first through the liver. But if the nitrogen balance becomes negative, amino-acids are thrown directly into the general circulation by the tissues, and may not reach the liver until after the particular portion of blood in which they are contained has circulated a number of times. It is quite possible that such circumstances might produce considerable changes in the quality of the amino-acid mixture circulating in the blood.

Finally, the deaminizing activity of the liver is probably influenced by the supply and demand of nutritive materials other than protein. In the case of a milking animal there is a large and steady demand for carbohydrate to be excreted with the milk. The supply of carbohydrate stored by mammals is normally rather small, and a shortage of this material in the food would, therefore, soon require the conversion of other compounds into carbohydrate to meet the demands of milk secretion. It has been repeatedly shown that carbohydrate is readily derived from protein in the animal economy (76, pp. 223–247), and it is very likely, therefore, that a shortage of carbohydrate in the food of a milking animal would soon stimulate the liver to increase its normal rate of deaminizing the products of protein digestion.

The three preceding paragraphs may be summed up by saying that general considerations make it likely that the quality as well as the quantity of the amino-acid mixture circulating in the blood is subject to change, and that both quality and quantity of the circulating amino-acid mixture are likely to be influenced by the supply of non-protein dietary constituents. There is already at hand a certain amount of experimental evidence which bears on these questions.

Delaunay (22), Van Slyke and Meyer (103), (104), Costantino (18), and György and Zunz (49) have shown that, during the digestion of a protein meal, the concentration of the amino-acid nitrogen in the blood is usually decidedly higher than when the intestinal tract is empty after 1 or 2 days' starvation. The earlier work of Folin and Denis (36), (37), (38), (39), (40), (41) shows the same thing, though these authors did not make any direct determinations of amino nitrogen.

Bang (4) has published results which lead him to conclude that the amino nitrogen of the blood is not, as a rule, increased by feeding protein. But the figures which he gives as representing amino nitrogen are really obtained by subtracting the urea nitrogen from the total non-protein nitrogen of the blood (4, p. 105), and, on account of this and of their general irregularity, they should hardly be taken as contradicting the mass of opposing evidence based on more direct methods.

Van Slyke and Meyer (104) have published figures for the amino nitrogen of the blood during several days' starvation. The results are rather irregular, but indicate, on the whole, that there is no marked decrease in the amino nitrogen of the blood during starvation continued for more than one or two days.

The work of Folin and Denis (36), (39) gives strong reason for believing that the quality of the amino-acid mixture contained in the blood of the general circulation is subject to alteration. These authors introduced various single amino-acids into the intestinal tract, and found that there was soon afterward a marked rise in the nitrogen fraction obtained by subtracting the urea nitrogen from the total non-protein nitrogen of the blood. It would be difficult to explain this result without supposing that the blood obtained during the absorption of the single amino acid contained much more of this latter body than was normal.

Three rather elaborate experiments have been carried out by Cary to throw light on the changes produced in the concentration and quality of the amino acid mixture of the blood by various kinds of changes in the diet of milking cows, and also on the manner in which milk yield is affected by changes in the amino acids of the blood. Abstracts of this work have already been published (15), and Mr. Cary has kindly given me permission to discuss the work further in this article.

All three experiments were begun with cows which were giving liberal quantities of milk, and which were on rations sufficient to provide for their maintenance and milk yield according to the figures given in the standard textbooks. Under these conditions, the composition and quantity of the milk and the concentration of amino-acid nitrogen in the blood and plasma were determined. The animals were then changed to rations which were inadequate in various respects, kept on these for varying periods, and finally changed back to the original adequate rations. Through all these changes, the composition and quantity of the milk and the concentration of amino-acid nitrogen in the blood and plasma were determined.

In the first experiment, the inadequate ration used contained only about half the required quantities of protein and total nutrients. In the second experiment, it contained half the required quantity of protein, but carbohydrate was substituted for the protein removed, so that it still contained the full requirement in total nutrients. In the third experiment, the inadequate ration contained the full requirement in protein, but only about half the original quantity of total nutrients. The change was accomplished chiefly by removing carbohydrate from the ration.

It will be possible, here, to give the results only in a very general way; those of the third experiment will be considered first. In this case, the reduction in the carbohydrate of the ration caused a reduction in milk yield, reductions in the amino-nitrogen content of the blood and plasma, and a reduction in the nitrogen content of the milk. The fat and sugar content of the milk remained practically unchanged. The results indicate that a shortage of carbohydrate in the food affects the milk yield through changes brought about in the amino-acid mixture of the blood.

It is not really surprising that this should be the case. A milking cow must have a large supply of carbohydrate daily to put into her milk. This material must come either from the carbohydrate of the food, from carbohydrate stored in the body, or from the conversion of some other material into carbohydrate. The available carbohydrate store is always small, probably not enough to supply a moderate milk yield for more than a few hours. When, therefore, the carbohydrate in the food of a milking cow is severely cut, the animal must soon begin to manufacture the missing material from something else. It seems quite reasonable to suppose that under such circumstances the liver would be stimulated to deaminize more rapidly the amino acids carried to it in the portal blood, and thereby to supply the missing carbohydrate and to reduce the concentration of amino nitrogen in the general circulation.

In view of the results of the experiment just described, it is not surprising to find that in the first experiment where the protein and total nutrients of the ration were reduced together, there was a very marked reduction in the amino-acid nitrogen of the blood, in the milk yield, and in the concentration of milk nitrogen. The milk sugar content was not determined in this experiment; the concentration of milk fat showed a general tendency to rise a little.

In the second experiment the quantity of protein in the ration was reduced, while the quantity of total nutrients remained unchanged. But, in this case, 50 per cent of the protein in the original adequate ration consisted of casein and lactalbumin, and 50 per cent consisted of vegetable protein. The reduction was made by leaving out all the casein and lactalbumin; and, as a consequence of this, the protein in the inadequate ration was not only reduced in quantity but also decidedly inferior in quality, as far as its relation to milk secretion was concerned. The result of the change in diet was that the quantity and nitrogen content of the milk were reduced, while the concentration of amino-acid nitrogen in the blood remained unchanged. In this experiment again, the sugar content of the milk remained unchanged on the inadequate diet. The fat content was decidedly decreased.

It is probable that the changes in quality of the food protein made by the change in diet brought about a corresponding change in the quality of the amino-acid mixture of the blood, and that this change produced the lowered milk yield and the lowered concentration of milk nitrogen, in spite of the fact that the concentration of total amino-acid nitrogen in the blood remained unchanged.

In a more recent experiment, the protein in the diet was reduced without changing its quality. In this case the same effects were obtained on milk yield as in the preceding experiment, and the concentration of amino-acid nitrogen in the blood was decidedly reduced.

When the animals in the experiments described above were changed back to the adequate rations, the changes in the milk yield and in the concentration of amino-acid nitrogen in the blood were not the exact reciprocals of those which were obtained by the change from the adequate to the inadequate rations. The reason for this is probably that the inadequate rations induced negative nitrogen balances, and that the animals were, therefore, in quite different nutritive condition at the beginning and end of each period on inadequate rations. It is unfortunately impossible to give any discussion of this aspect of the experiments here.

The most important general conclusions to be drawn from the experiments which have just been described are that both the quantity and the quality of the amino-acid mixture circulating in the blood are changed by changes in diet, that these changes have a marked influence on milk secretion, and that protein metabolism is intimately related to the metabolism of both carbohydrate and fat. A change in the carbohydrate of the diet may affect the amino-acids of the blood, and thereby the secretion of milk protein; and a change in the protein of the diet may affect the secretion of milk fat—probably again through a change in the amino-acids of the blood.

2. Relation between milk secretion and the carbohydrate, phosphatid and calcium of the blood. No experiments have been carried out in which the milk yield and either the dextrose, phosphatid or calcium of the blood have been studied simultaneously. But there is a good deal of evidence in regard to how the concentration of these three constituents of blood varies under various physiological conditions; and, also. as to how variations in the quantity of carbohydrate, fat, phosphorus and calcium in the food affect the milk yield. Bringing these two sets of investigation into relation with each other throws a good deal of light on the physiology of nutrition and of milk secretion.

The concentrations of both dextrose and phosphatid in the blood are subject to considerable variation. The blood sugar content may vary with the quantity of carbohydrate supplied in the food (77, p. 223), and is also altered by such influences as anxiety, pain and anesthesia (13, pp. 66–80) (100). The concentration of milk sugar, on the other hand, is surprisingly constant (89), (90), (91), (92), (25), (15). None of the investigations so far carried out throws any light on the question whether milk secretion is affected by changes in the concentration of blood sugar. In Cary's investigation (15) the milk yield was found to decrease when the carbohydrate in the ration was greatly decreased. But Cary's work shows that a reduction in the carbohydrate of the food causes a reduction in the concentration of amino nitrogen in the blood and also a reduction in the concentration of the milk nitrogen. It is quite probable that changes in the amount of carbohydrate supplied in the food, and changes in carbohydrate metabolism generally, affect the milk yield through changes which they induce in protein metabolism rather than through any change brought about in the concentration of carbohydrate in the blood. The same comment would apply to a number of researches in which the effects of phlorhidzin administration on milk yield and on the concentration of milk sugar were studied (17), (19), (93), (94), (95).

The concentration of phosphatid in the blood has been shown to be increased by feeding fat (9), (10). It is also increased during milk secretion independently of the food supply (84). The work of Morgen and his collaborators (89), (90), (91), (92) shows that the concentration of milk fat may be influenced by the fat of the food; that of Jordan, Hart and Patten (69), that it may be influenced by the phosphorus contained in the food. It seems likely that changes in the quantity of fat or phosphorus contained in the food influence the secretion of milk fat by bringing about changes in the concentration of phosphatid circulating in the blood; but as yet no direct proof of this proposition has been adduced.

The concentration of calcium in blood plasma is more constant than that of any other consitutent which has been hitherto studied. In adult human beings and cattle the normal limits are from 9 to 11 mgm. per 100 cc. plasma (51), (64), (66), (72), (73), (74), (82), (84), (87). The changes in concentration which can be brought about by changing the quantity of calcium in the food of these animals are barely outside the limits of error for the determinations (8), (50), (84). It is not surprising, therefore, to find that reductions in the amount of calcium in the food, even when sufficient to bring about a marked negative calcium balance, have no immediate effect on milk secretion (33), (42), (43), (44), (52).

It will be seen from the foregoing paragraphs that our knowledge of the manner in which diet affects milk secretion through changes brought about in the concentrations of the various milk precursors in the blood is still in a very fragmentary state. The evidence now on hand seems to justify the following views.

Milk secretion is preëminently affected through changes in the quality and quantity of the amino-acid mixture circulating in the blood. Such changes are brought about by changes in the quantity or quality of the protein fed, and also by marked changes in the quantity of the non-protein portion of the ration. They tend to affect the whole amount of milk yielded rather than the concentration of protein in the milk, though the latter kind of change can easily be detected when the experimental conditions are appropriate. The amino-acid mixture circulating in the plasma seems, under certain circumstances, to have an effect on the secretion of milk fat.

In the experimental procedures hitherto used the effects on milk secretion of changes in carbohydrate metabolism are confused with the effects of changes induced in protein metabolism. It is improbable that milk secretion is affected by any changes in the concentration of blood sugar which can be brought about under ordinary circumstances.

Milk fat is derived from the phosphatid of the blood plasma. Its secretion is probably, to some extent, dependent on the concentration of phosphatid in the blood, and therefore on both the fat and phosphorus supplied in the food. That milk fat is usually derived from food fat is shown not only by the work of Morgen and his collaborators frequently referred to above, but also by numerous investigations in which it has been shown that the quality of the milk fat is influenced by the quality of the food fat and that fatty acids not normally contained in butter can be made to appear in it if they are supplied in the food. As examples of the evidence for these two propositions, the work of Eckles and Palmer (26) and of Bowes (12) may be referred to. These authors give references to previous work on the subject. The connection between the secretion of milk fat and the phosphorus of the food is shown by the work of Jordan, Hart and Patten (69).

The work of these authors shows that the concentration of milk phosphorus is not affected by the phosphorus supplied in the food, though the concentration of milk fat is affected; and the reason for this rather peculiar situation is given in the work of Meigs, Blatherwick and Cary (84). The phosphatid of the blood contains more phorphorus in proportion to fat than does the milk. In taking sufficient phosphatid from the blood to provide for the milk fat, therefore, the mammary gland always gets more phosphorus than is required to go into the milk, and it returns the surplus to the blood as inorganic phosphate. It is quite conceivable that the return of this extra phosphorus to the blood might be independent of the amount of phosphorus contained in the food, and that the concentration of phosphorus in the milk might, therefore, be independent of the food phosphorus.

The calcium contained in milk must be derived from the calcium of the blood plasma or from some part of it. The plasma calcium may, therefore, be regarded as the precursor of the milk calcium. The concentration of plasma calcium is extremely constant and largely independent of the food supply. It is not surprising, therefore, to find that changes in the quantity of calcium in the food have no immediate effect on milk secretion. A long-continued deficiency of calcium and phosphorus in the food does finally bring about a reduction in milk secretion, as has been shown by Fingerling (33). But the same author shows that, as the total yield of milk falls off, the concentration of calcium and phosphorus therein rises; and it seems quite possible that a deficiency of calcium in the diet may affect milk yield in some indirect way, and not through a change in the calcium content of the blood plasma.

IV. QUANTITATIVE ESTIMATIONS OF THE AMOUNTS OF PROTEIN AND ENERGY REQUIRED FOR MILK SECRETION. An account of the relation between milk secretion and diet would be incomplete without a brief review of the attempts which have been made to determine the quantities of protein and total nutrients or energy required to support a given amount of milk secretion in cows.

Accounts of the work along these lines and references to the literature are given in the standard textbooks on cattle feeding (3), (60), (71), (75), and tables are published in which it is stated that a cow of a given weight requires a certain quantity of protein and a certain quantity of total nutrients for her maintenance, and, in addition, so much protein and so much nutrients for each pound of milk with a given fat content.

It is clear from a study of the original work on the subject that the aim has been to determine the quantities of protein and nutritive energy necessary to keep a cow in nutritive equilibrium when she is giving a certain quantity of milk. The difficulties in the way of making such determinations are very considerable. It requires, in the first place, an elaborate and expensive experiment to determine whether an animal is in nitrogen equilibrium or in energy equilibrium: many of the investigators of the subject have not attempted to do this accurately, but have merely used the body weight as a rough indication as to whether their animals were in nutritive equilibrium or not. Further difficulties are introduced by the natural variation in the qualities of foods, and in the different proportions of the same digested by different animals or by the same animal under different conditions. In the case of protein, finally, there is, strictly speaking, no such thing as a quantitative requirement for milk secretion at all. The milk proteins contain certain amino-acids, such as tryptophane and lysine, which cannot be manufactured from other materials by the animal body and which are contained in varying amounts in certain vegetable proteins and are absent altogether from others (105, pp. 70 et seq.). The quantity of protein required to support a given milk yield will vary, therefore, with the quality of the protein supplied; and it is obvious that the figures given as the protein requirement for milk secretion cannot be taken as generally accurate for all kinds of protein. They represent, at best, the requirement for milk secretion of the particular kind of protein used in the experiment in which they were obtained.

In spite of all these difficulties, the figures obtained by different investigators do not vary so widely as might be supposed. As was to have been expected, the figures given for protein requirement are the most variable. It is difficult to make a thorough-going comparison of all the figures, because the different investigators give their results in different terms which are not strictly comparable. In a general way, however, it may be said that the difference between the high and low figures given for protein requirement amounts to about 33 per cent of the latter; while the same difference in the case of total nutrients or energy amounts to about 13 per cent (3, p. 714; 60, pp. 133 and 667). If the figures for the requirements are compared with the products obtained in the milk, it will be found that the production of a gram of protein or of total nutrients in the milk requires about twice that weight of protein or of total nutrients in the food, in addition to the maintenance requirement (60, p. 133).

The optimum yield of a milking animal is often considerably above that which she will give when kept in nutritive equilibrium. A clear discussion of this phase of the subject is given by Armsby (3, pp. 513 et seq.). If fed more than is required to preserve nutritive equilibrium, the lactating animal will put part of the surplus into an increased energy output, part into body growth, and part into increased milk yield. Just what proportion of the increased food intake will take each of these three outlets under different circumstances is a question of very great practical importance, but there are considerable difficulties in the way of its investigation, and it has so far hardly been touched.

V. VITAMINES AND MILK SECRETION. The question of the relation of vitamines in the diet to milk secretion has, as yet, been little studied.

The whole subject is too new to make possible any very detailed discussion or definite conclusions, but there are not wanting results which point to its importance, and these will be briefly referred to. An extensive review of the general subject of vitamines in nutrition is given by Sherman (100a).

It is probably a mistake to suppose that the three little known materials which at present occupy the center of the stage under the name of vitamines are the only unknown chemical compounds which are required for the nutrition of all animals under all physiological conditions. We already have hints of the existence of one or two rivals, and it is altogether probable that a good many more will make their appearance as soon as investigators begin to search for them. For some time past evidence has been in existence which indicates that there are compounds which have an influence on milk secretion in addition to those generally recognized.

The evidence in question comes from the work of Morgen and his collaborators (32), (91). These investigators found that when milking animals were fed on a basal ration of factory products, their milk yield was noticeably increased by the addition of small quantities of what they call "Reizstoffe"—certain aromatic seeds and the water extract of meadow hay. The basal rations in these experiments were probably low in all three vitamines, and the beneficial effects on milk yield of adding the "Reizstoffe" may have been due to the addition of either the water-soluble or the fat-soluble vitamine, or of both. It is possible, on the other hand, that they were due to some material not identical with any of the three vitamines which are receiving so much consideration at present.

According to McCollum (79), the fat-soluble vitamine is generally represented fairly well in the leaves of plants, while the water-soluble is everywhere plentifully present in seeds. Most cattle rations composed of grain and hay ought, therefore, to contain these two vitamines.

It seems likely, however, that milking animals kept on the usual winter rations would get very little of the antiscorbutic vitamine in their food. Barnes and Hume (5) have reported that the antiscorbutic property of cow's milk varies with the season, and Hart, Steenbock and Ellis (58) have published experiments showing that the milk from cows on pasture contains more of the antiscorbutic vitamine than that from cows fed exclusively on winter rations. These results have been confirmed by Hess (62) and by Dutcher and others (23). They indicate that the antiscorbutic vitamine is not synthesized by milking animals, and that its absence from the food reduces its concentration in the milk rather than the total milk yield.

McCollum, Simmonds and Pitz (78), (80) have described experiments which indicate that the growth of young nursing rats is retarded when the fat-soluble and water-soluble vitamines are absent from the diet of their mothers. The authors believe that this is due to a reduced concentration of the vitamines in the milk rather than to a reduction of the total milk yield, but they adduce no experimental evidence in support of their belief. Dutcher (23), however, and Hughes (65) have announced that in experiments to be published later they have shown that it is possible to reduce the concentration of either the fat-soluble or the water-soluble vitamine in milk by withholding these materials from the food of the nursing mother.

Hart, Steenbock and Hoppert (59) find that milking goats fed the fresh green oat plant assimilate calcium better than when fed the same plant in the dried state, and they attribute the better calcium assimilation to a vitamine contained in the fresh material. The connection between milk secretion and calcium assimilation is so important that these experiments will be considered here, although they do not bear directly on the relation between vitamines and milk secretion.

Hart and his collaborators carried out further experiments directed toward determining the nature of the material which is contained in the green oat plant, and which facilitates calcium assimilation. They determined the calcium balance in animals which were fed on a basal ration of dried materials with additions of cabbage, orange juice, butterfat or cod-liver oil. The cod-liver oil was the only one of these materials which facilitated calcium assimilation. The authors consider the results with cabbage and orange juice sufficiently definite to show that the antiscorbutic vitamine has no favorable influence on calcium assimilation. They do not think that the results with butter fat are conclusive, and they leave open, therefore, the question whether the material which facilitates calcium assimilation is the fat-soluble vitamine or some unknown material contained in cod-liver oil. The question whether absence of the fat-soluble vitamine from the diet may be a cause of the faulty calcium assimilation characteristic of rickets has been studied by a number of investigators with rather varying results (61), (63), (81), (85), (86), (101).

The work on the relation between milk secretion and the vitamine content of the diet, which has just been reviewed, may be summarized by saying that the evidence so far obtained tends to indicate that changes in the vitamine content of the diet influence directly the concentration of vitamines in the milk rather than the amount of milk secreted. Whatever influence the vitamines of the food may have on milk yield is probably indirect and therefore more or less delayed. The field, however, is too new to justify the drawing of any very positive or detailed conclusions.

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THE FATE OF FOREIGN ORGANIC COMPOUNDS IN THE ANIMAL BODY

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Due to the fact that it is utterly impossible in an article of two or three dozen pages to consider any particular part of so vast a subject in detail, generalities alone will be emphasized. Furthermore, as the references which bear directly on the matter number at present over two thousand with perhaps as many more which are very closely related to this type of work, we have been obliged to limit the bibliography to those references which are the more important. Heffter (37) in his Ergebnisse article has covered the literature quite fully up to the year 1904. One is also referred to Frankel's excellent work *Arzneimittelsynthese* (31) particularly for a discussion of the subject from the standpoint of pharmacology.

Foreign organic compounds introduced into the animal body by way of the gastro-intestinal tract or by subcutaneous or intravenous injections can not be considered as foods but must be treated under the collective heading of poisonous or toxic substances. The problem, therefore, is one of toxicology rather than of normal physiology or physiological chemistry, and resolves itself into a study of the "chemical defense mechanism of the animal organism." The animal body, in order to protect itself from these toxic materials, must be able either to destroy them completely or, if this is impossible, to detoxicate them in one way or another and eliminate them in one of the body's excretions; and as the urine is the usual channel of elimination, the solubility of the detoxication products becomes a very important factor.

To attain these ends the body has at its command a series of chemical reactions—reactions, by the way, which we are unable at the present time to duplicate in our best equipped laboratories. The first method of attacking a foreign molecule seems to be an attempt at *complete oxidation.* This, in the majority of cases meets with at least partial success. If the compound be an aliphatic fatty acid its destruction will likely be complete; if it be an aromatic derivative of a long chain acid the side chain will likely be reduced to one or two carbon atoms, depending, as we shall see later, on the number of carbons composing the original chain. In some cases *reduction* is used as a precursor of oxidation, and in a few instances as an independent reaction. Should the foreign molecule be able to withstand these types of attack, or yield to them at most only partially, it is necessary for the body to have recourse to a synthetic type of reaction, combining the toxic substance with some radical or molecule which it has at its disposal. In this way not only is the toxicity reduced but the resulting product is rendered more soluble. This synthetic type of reaction embraces both the *conjugation* with one of the amino acids such as glycocoll, glutamine or cysteine, or perhaps with sulphuric or glycuronic acids; as well as *methylation*, *acetylation* and *uramino acid formation*.

Knoop (52) noticed that whenever an aromatic derivative of a fatty acid was fed to an animal it appeared in the urine either as benzoic acid or phenylacetic acid in combination with glycocoll, i.e., as hippuric or phenaceturic acids. Further consideration showed that the benzoic acid formers were invariably those containing an odd number of carbon atoms in the chain, such as phenylpropionic or phenylvaleric acids, while those acids like phenylbutyric and phenylcaproic, containing an even number of carbon atoms in the side chain always produced phenylacetic acid. No acid with an odd number of carbon atoms in the chain ever furnished an intermediary product of metabolism with an even number of carbons. Accordingly he concluded that these compounds must be shortened by the splitting off of two carbons at a time, that is to say, the β-carbon was in each case the target for oxidation. This view was strongly supported by the work of Dakin (18) who detected β-phenyl-β-hydroxy-propionic acid in the urine after feeding phenylpropionic acid.

$$C_6H_5\cdot CH_2\cdot CH_2\cdot COOH \ldots\ldots\ldots\ldots\ldots C_6H_5\cdot CHOH\cdot CH_2\cdot COOH$$

Phenylpropionic Acid β-Phenyl-β-hydroxy-propionic Acid.

It has been pointed out, moreover, by various investigators that the natural fats are composed of fatty acids consisting of an even number of carbon atoms and that these fats in the organism of the diabetic are the precursors of β-hydroxy-butyric acid. Likewise the unsaturated side chain aromatic acids apparently undergo β-oxidation. Thus cinnamic acid, $C_6H_5\cdot CH{:}CH\cdot COOH$, is oxidized to benzoic acid (27) in the animal body to form β-phenyl-β-hydroxy-propionic acid (21) as an intermediary product.

Amino acids, particularly aliphatic α-amino acids, $R\cdot CHNH_2\cdot COOH$, are apparently subject to attack at the α-carbon position, and either by oxidative or hydrolytic deamination are converted into α-hydroxy, $R\cdot CHOH\cdot COOH$, or α-keto, $R\cdot CO\cdot COOH$, fatty acids. In either case but one carbon atom is split off, and the α-carbon is oxidized to a carboxyl group. The fatty acid thus formed is then subject to the general rule of β-oxidation.

Alcohols of the aliphatic series are oxidized to the corresponding acids, for example, methyl alcohol to formic acid (80). Primary and secondary alcohols as a rule are easily oxidized in the organism, but tertiary alcohols are apparently burned with great difficulty. Thus ethyl alcohol in small amounts is completely changed to carbon dioxide and water, and isopropyl alcohol, $(CH_3)_2CHOH$, is largely converted into acetone $(CH_3)_2CO$ (5), whereas tertiary alcohols are usually excreted in combination with glycuronic acid, as was found for tertiary amyl alcohol, $(CH_3)_2COH\cdot CH_2\cdot CH_3$, and tertiary butyl alcohol, $(CH_3)_3$-COH. The halogen substituted alcohols are as a rule excreted in combination with glycuronic acid and seem particularly resistant to oxidation (71). Two well-known cases are trichlorethyl alcohol, $CCl_3\cdot CH_2OH$, (70) and trichlorbutyl alcohol. Aldehydes are seldom if ever found as intermediary products of alcoholic oxidation, but conversely the reduction of aldehydes to alcohols as chloral, $CCl_3\cdot CHO$, to trichlorethyl alcohol, $CCl_3\cdot CH_2OH$, is by no means rare.

The dicarboxylic acids are in general more resistant to oxidation than the fatty acids. Thus oxalic acid, $(COOH)_2$, according to some authors is not oxidized at all (66); however, when malonic acid, $COOH\cdot$-$CH_2\cdot COOH$, is fed, relatively small amounts of the unoxidized material appear in the urine, while traces of it are excreted as oxalic acid. Succinic acid, $(COOH\cdot CH_2)_2$, and glutaric acid, $COOH\cdot(CH_2)_3\cdot COOH$, are quite easily burned by the organism. It would seem from this that the resistance to oxidation of this series of acids decreases with rise of molecular weight, following much the same rule as that laid down for physiological oxidation of the fatty acids, namely, that the possibility of oxidation varies indirectly with the volatility of the member of the series. Both rules, however, have many exceptions, particularly the one regarding the dicarboxylic acids. Thus adipic acid is much harder to oxidize than any of the lower members of the series.

Some of the lower hydroxy, aldehyde and keto acids are important on account of their relationship to the probable intermediary products of carbohydrate metabolism. Glycollic acid, $CH_2OH\cdot COOH$, and glyoxylic acid, $CHO\cdot COOH$, are both oxidized in a large measure to oxalic acid, $(COOH)_2$, (16). Pyruvic acid, $CH_3\cdot CO\cdot COOH$, administered in small doses, appears to be completely oxidized, whereas, after subcutaneously injecting into a cat 7 grams of the substance as the sodium salt, (25), there appeared in the urine of the animal glucose, dl-lactic acid and the sodium salt of some unchanged pyruvic acid.

The amides of the higher fatty acids are easily hydrolyzed into ammonia and the corresponding fatty acids, the ammonia fraction going to increase the urea output while the fatty acid is subject to decomposition according to the rule of β-oxidation. The amides of the lower fatty acids, on the contrary, such as acetamide (25), $CH_3\cdot CONH_2$, appear to be very resistant to oxidation and pass through the organism unchanged. The α-amino acids found in protein, of the type $R\cdot CHNH_2\cdot COOH$, are completely destroyed in the animal body after an initial process of oxidation or hydrolytic deamination, which shows that the amino group is the most sensitive part of the molecule. It is interesting to note that while the optical antipodes of these amino acids are usually treated by the body as non-toxic foreign substances and excreted unchanged in the urine, the dl-forms of some of the lower amino acids are completely oxidized, for example, dl-alanine, $CH_3\cdot CHNH_2\cdot COOH$ (33).

A number of investigators have substituted one or both of the hydrogen atoms of the amino group of these acids and fed them, with the result that they were found to be very resistant to oxidation. Friedmann noted that when the amino acids found in proteins were monomethylated, $R\cdot CH(NH\cdot CH_3)\cdot COOH$, the lower members of the series were excreted free to the amount of about one-third of the total that was fed, while the higher members were excreted almost quantitatively. A number of the amino acids have been benzoylated (65), $R\cdot CH(NH\cdot CO\cdot C_6H_5)\cdot COOH$, or phenylacetylated (96), $R\cdot CH(NH\cdot CO\cdot CH_2\cdot C_6H_5)\cdot COOH$, with the result that they also were excreted in the free state.

The unsaturated aliphatic acids are completely oxidized in the body as is shown in the case of acrylic acid, $CH_2{:}CH\cdot COOH$, (63). The corresponding aromatic acids, however, are oxidized only to benzoic or phenylacetic acids.

$$C_6H_5\cdot CH{:}CH\cdot COOH \rightarrow C_6H_5\cdot COOH$$

Cinnamic Acid. Benzoic Acid.

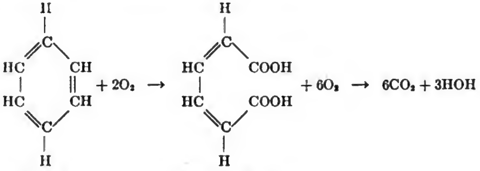
$$C_6H_5\cdot CH_2\cdot CH{:}CH\cdot COOH \rightarrow C_6H_5\cdot CH_2\cdot COOH$$

Phenylisocrotonic Acid. Phenylacetic Acid.

The γ-substituted fatty acids are mostly excreted unchanged, but a few are converted into their lactone forms. Thus phenylbutyric acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$, is excreted as its lactone. An interesting exception to this common rule is the case of phenyl-γ-keto-butyric acid, $C_6H_5 \cdot CH_2 \cdot CO \cdot CH_2 \cdot COOH$. In this case the carbonyl group is apparently reduced and the phenylbutyric acid thus formed is then oxidized to phenylacetic acid and excreted in combination with glycocoll (phenaceturic acid).

In the oxidation of aromatic substances many complications are met with. In a simple case like the phenyl derivative of a fatty acid there is mere β-oxidation of the side chain, but where there are two or more side chains or substitution products so many new factors appear that these cases are best dealt with separately. In general the benzol ring is very resistant to the oxidizing forces of the body, so much so, in fact, that the breaking of this ring is found in exceedingly few cases. The notable cases are those acids possessing a chain of three carbon atoms, one end of which chain is attached to the benzol ring while the center carbon holds an amino group (6),(7),(13),(91),(92), as phenylalanine, $C_6H_5 \cdot CH_2 \cdot CHNH_2 \cdot COOH$, or tyrosine, p-$OH \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$ or α-aminocinnamic acid, $C_6H_5 \cdot CH{:}CHNH_2 \cdot COOH$.

Again, isophthalic acid, $C_6H_4 \cdot (COOH)_2$ (1–3), and terephthalic acid, $C_6H_4 \cdot (COOH)_2$ (1–4), are said, to some extent at least, to be completely oxidized in the body (82), while the o-compound, $C_6H_4 \cdot (COOH)_2$ (1–2), phthalic acid itself, is not subject at all to oxidation (81). After the feeding of o-nitro-benzaldehyde to dogs Cohn (14) found only about 10 per cent of the material excreted unchanged and believes that the remainder was oxidized in the body. Sherwin and Hynes (95) obtained much the same results after feeding the compound to dogs and rabbits, but when human subjects were employed, 80 per cent of the ingested material was recovered as o-nitrobenzoic acid. This negative sort of evidence hardly justifies one in making the assertion that this is another case where the o-compound is easily destroyed in the body while the m- and p-compounds remain unoxidized (32). In this case, as in many others, the o-compounds appear to be so much more toxic than the m- and p-compounds that they must be fed in much smaller doses. Accordingly they may be lost in the attempt to isolate them or may be handled by the body in some as yet undiscovered way and thus escape detection in the urine. The most interesting compound is benzol itself, but there is so much conflicting evidence regarding its fate in the body that the entire question should be reinvestigated. Jaffé (48) discovered an unsaturated acid in the urine of animals after feeding benzol and was able to isolate muconic acid. On injecting muconic acid into rabbits in amounts as large as 2 grams he was able to recover only 1 per cent of the substance unchanged. From this work one is led to believe that about 25 to 30 per cent of the ingested benzol was split into muconic acid, but the muconic acid being an easily oxidizable substance was further burned to water and carbon dioxide leaving only a trace of the intermediary product of metabolism for excretion. If this be the case the reaction is expressed by the following simple equation:



Benzol. Muconic Acid.

There is a very close relationship between the saturated and unsaturated acids insofar as their physiological ozidation is concerned. It seems therefore rather improbable that muconic acid should be so easily oxidized when the corresponding saturated acid, adipic acid, is oxidized with so great difficulty.

$COOH\cdot CH:CH\cdot CH:CH\cdot COOH$ $COOH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot COOH$

Muconic Acid. Adipic Acid.

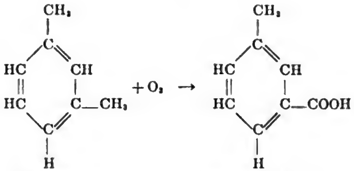
With this point in view, Mori (73) repeated the work of Jaffé and was unable to substantiate his claims. On the contrary, Mori found that after injecting 0.8 gram muconic acid into rabbits 73 per cent of the substance, on the average, was excreted unchanged, and adipic acid injected in the same quantities, was eliminated as such to the amount of 61 per cent. After administering adipic acid there was always an increase in oxalic acid excretion as a result of the β-oxidation of some of the adipic acid, but not even this evidence of oxidation was noted in the case of muconic acid. Not only does this destroy our most plausible explanation for the oxidation of the aromatic nucleus in the body but it also excludes the probability of the existence of muconic acid as an intermediary metabolic product of benzol.

It has been stated above that α-amino-β-phenylpropionic acid (phenylalanine) and some of its derivatives are completely burned in the body. Tyrosine, which is phenylalanine with an -OH group in the p-position, is seemingly burned with as much ease as phenylalanine itself. If, however, the position of the -OH group in the ring be changed, i.e., if o- or m-hydroxyphenylalanine instead of the p-hydroxy compound be fed, there is a very slow and incomplete oxidation, resulting so far as it proceeds, in the formation of o- or m-hydroxyphenylacetic tic acid (11), (28). M-chlorphenylalanine as well as the p-chlor compound have been found to yield the corresponding phenylacetic acids (34). Dakin (20), however, found that both p-methylphenylalanine, $CH_3 \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$, and p-methoxyphenylalanine, $CH_3O \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$, were easily and completely oxidized in the body of patients suffering from alkaptonuria. This, with other instances (12), signifies that a methyl ($-CH_3$) group in the benzol ring does not act as a hindrance to the oxidation of the ring. A chlorine atom, however, in any position proves a block to further oxidation rather than a sensitive point at which the ring may be split.

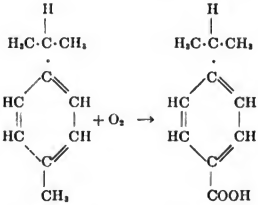
Perhaps the most resistant to oxidation are the phenyl substitution products of the fatty acids. These as we have previously seen are first oxidized to benzoic or phenylacetic acids, then combined in most cases with glycocoll to form the corresponding hippuric or phenaceturic acids. Not only is this true of the long chain phenyl derivatives of the fatty acids but it also holds for most of the substitution products of these acids where one or two hydrogen atoms in the ring have been replaced by a nitro group, an amino group, a hydroxy group or one of the halogens.

Where there have been substitutions in the side chain of the acid, oxidation of this side chain is either hindered or facilitated, depending first, on the number of carbons in the chain, and secondly, on the positions and kinds of substituted groups. We have previously seen that aromatic acids with three carbon atoms in the side chain, containing also an α-amino group, for example, α-amino-β-phenylpropionic acid, are completely oxidized in the animal body. This is likewise true of certain α-hydroxy and α-keto acids, such as β-phenyllactic acid, $C_6H_5 \cdot CH_2 \cdot CHOH \cdot COOH$, and phenylpyruvic acid, $C_6H_5 \cdot CH_2 \cdot CO \cdot COOH$. If, however the amino, hydroxy or keto group occupies a β-position, oxidation is incomplete, for the side chain alone is attacked. Thus β-phenyl-β-aminopropionic acid, $C_6H_5 \cdot CHNH_2 \cdot CH_2 \cdot COOH$, and β-phenyl-β-hydroxypropionic acid, $C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$, are both oxidized only to benzoic acid, while benzoylacetic acid, $C_6H_5 \cdot CO{:}CH_2 \cdot COOH$, (19), is partly excreted as such and partly as acetophenone, $C_6H_5 \cdot CO \cdot CH_3$, together with some β-phenyl-β-hydroxypropionic acid, $C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$, and some cinnamic acid, $C_6H_5 \cdot CH{:}CH \cdot COOH$, the latter in combination with glycocoll. This a reduction without β-oxidation, found in the β-amino or β-hydroxy acids. Phenylacetic acid also forms α-amino, α-hydroxy and α-keto compounds. If d-phenylglycine, $C_6H_5 \cdot CHNH_2 \cdot COOH$, is fed, mandelic acid, $C_6H_5 \cdot CHOH \cdot COOH$, is excreted, probably with the formation of phenylglyoxylic acid, $C_6H_5 \cdot CO \cdot COOH$, as an intermediary product (77). When, however, dl-phenylglycine is ingested, there appears in the urine chiefly l-phenylglycine along with dl-acetylaminophenylglycine, $C_6H_5 \cdot CH \cdot (NH \cdot CO \cdot CH_3) \cdot COOH$, l-mandelic acid and glyoxylic acid (78). The three latter substances are degradation products of the d-phenylglycine. A small fraction of both the d- and the l-compounds is oxidized to benzoic acid. Phenylglyoxylic acid when fed to dogs or human beings is reduced to l-mandelic acid. From the literature it is difficult to decide whether the l-mandelic acid alone is formed or whether both the d- and the l-forms are produced but with different pathways of elimination. Schempp (89) found only dl-mandelic acid in the urine of men and dogs after feeding phenylbromacetic acid, ($C_6H_5 \cdot CHBr \cdot COOH$). Since simple hydrolysis of this compound, however, takes place even in moist air, Schempp's finding is of little physiological importance. The separation of dl-substances into their active components with subsequent oxidation of one and total elimination of the other has been noted by various experimenters. Thus dl-p-hydroxyphenylglyoxylic acid, $4\text{-}OH \cdot C_6H_4 \cdot CHOH \cdot COOH$, when fed to rabbits (24), is split into its optical antipodes followed by oxidation of the l-component and excretion of the d-form.

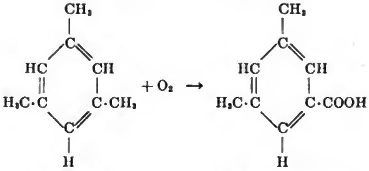
Where more than one side chain is attached to the benzol ring, oxidation is usually confined to but one of these chains (74), (75), (76), (93). A few of the best known cases are those of m-xylene, p-cymene, mesitylene and pseudo-cymene. All of these are oxidized on one side chain to the corresponding acids:



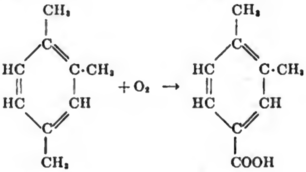
m-Xylene. m-Toluic Acid.



p-Cymene. Cumic Acid.

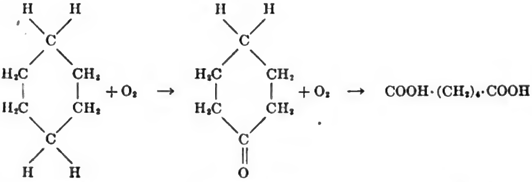


Mesitylene. Mesitylenic Acid.



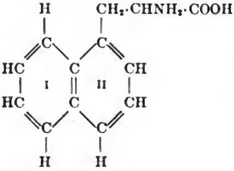
Pseudo-cymene. Xylic Acid.

Cyclohexane is oxidized in a large measure to cyclohexanone and small amounts even further with ring splitting to adipic acid:

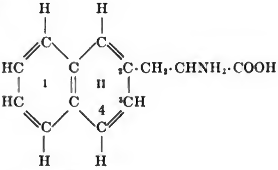


Cyclohexane. Cyclohexanone. Adipic Acid.

The naphthalene nucleus is also partially destroyed in the organism as was demonstrated by the interesting experiment of Kikkoji (51). He wished to compare the fate in the body of a double ring compound containing a side chain of three carbon atoms holding also an α-amino or α-keto group, with that of phenylalanine. He prepared two different compounds, α-naphthylalanine and β-naphthylalanine and fed them to dogs.

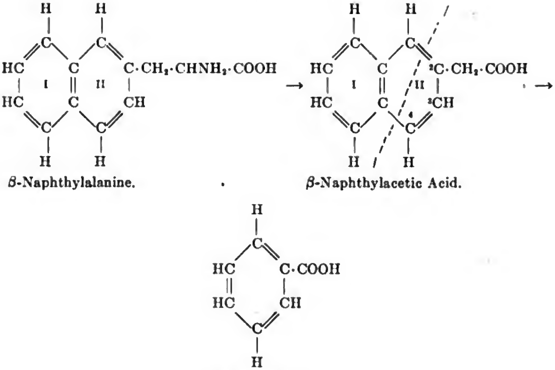


α-Naphthylalanine.



β-Naphthylalanine.

From the urine, after feeding the α-compound, he was able to isolate an unknown derivative of naphthylene which apparently had both rings intact. Nothing could be found that indicated that either ring had been split. After feeding β-naphthylalanine, however, he found first that the side chain had been deaminized and shortened to two carbon atoms, then that a second reaction had split ring II with the formation of benzoic acid which was excreted as hippuric acid. The reaction is seemingly the one pictured below:



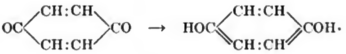
β-Naphthylalanine.

β-Naphthylacetic Acid.

Benzoic Acid.

He concludes from this that α-amino acids are shortened by one carbon at a time and that the splitting of the ring occurs between carbon atoms 1 and 2 instead of between 3 and 4; else the resulting compound would have been phenylbutyric acid, $C_6H_5 \cdot (CH_2)_3 \cdot COOH$, which in turn would have been oxidized to phenylacetic acid, $C_6H_5 \cdot CH_2 \cdot COOH$, and have been found in the urine as phenaceturic acid.

Reduction. Reduction in the animal body, while not so common as oxidation, is by no means of rare occurrence. One of the first cases found in the literature is that of the reduction of chloral, $CCl_3 \cdot CHO$, to trichlorethyl alcohol, $CCl_3 \cdot CH_2OH$, (58), (59), (70), (71),—a reduction, it may be remarked, which is brought about in the laboratory only with difficulty. Other reductions have since been found, such as that of quinone to hydroquinone:

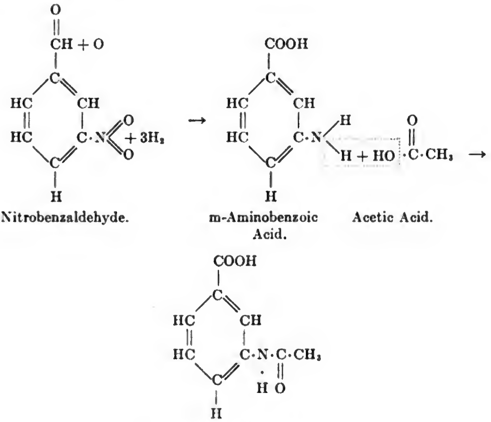


$$OC\begin{array}{c} CH:CH \\ CH:CH \end{array}CO \rightarrow HOC\begin{array}{c} CH:CH \\ CH:CH \end{array}COH.$$

Unsaturated acids are changed in the organism into saturated compounds, carbonyl (-CO) groups into secondary alcohols (-CHOH), and both may even be reduced to methyl groups ($-CH_3$), (56) (100).

After feeding o-nitrotoluene, Jaffé (43), (46) found that the compound had been oxidized partly to o-nitrobenzyl alcohol and partly to o-nitrobenzoic acid. In this case there was very probably an oxidation to the aldehyde with a simultaneous oxidation and reduction analogous to the Cannizzaro reaction, $R\cdot CHO + R\cdot CHO \rightarrow R\cdot CH_2OH + R\cdot COOH$.

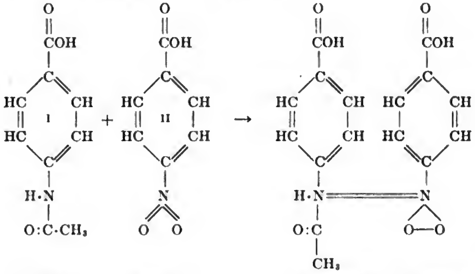
There is in the literature a number of cases where oxidation and reduction occur simultaneously within the same molecule. A remarkable case of this kind was noted by Cohn (14) after feeding m-nitrobenzaldehyde to rabbits:



m-Nitrobenzaldehyde. m-Aminobenzoic Acid. Acetic Acid.

m-Acetylaminobenzoic Acid.

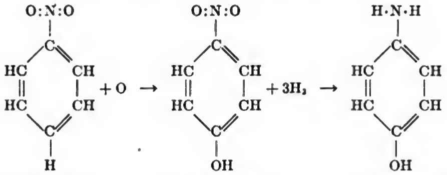
Here we see the simultaneous oxidation of an aldehyde (-CHO) group to a carboxyl (-COOH) and the reduction of a nitro group to an amino. As a secondary reaction we have the interaction between the m-aminobenzoic acid thus formed and an acetic acid molecule resulting in the formation of an acetylamino compound. After feeding the p-nitrobenzaldehyde to rabbits there is formed in an analogous manner, p-acetylaminobenzoic acid from a part of the substance, while another part is merely oxidized on the aldehyde group to p-nitrobenzoic acid. Then to complicate matters still more there is a combination of these two molecules through their nitrogen atoms (84).



p-Acetyl-aminobenzoic Acid. p-Nitro-benzoic Acid. Combination of molecules I and II

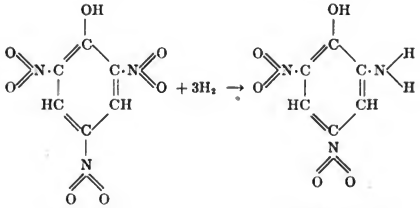
In contrast to the above mentioned nitrobenzaldehydes the o-nitrobenzaldehyde is excreted as o-nitrobenzoic acid only to about 10 per cent of the amount fed. The remainder has thus far escaped detection. It is interesting to note here that the nitrobenzaldehydes act much differently in the organisms of the dog (99) and the human being (95). The o-nitro compound, when fed to dogs, also disappears in amounts as great as 90 per cent, while from the human organism it has been recovered in the form of o-nitrobenzoic acid in relatively large amounts. Both the m-nitro and the p-nitro benzaldehydes, instead of being reduced, in either case are oxidized to the corresponding acids and excreted to a large extent in combination with glycocoll, i.e., as m-nitro and p-nitro hippuric acids. Again, p-nitrophenylacetaldehyde, when fed to a human being, a rabbit and a dog, was merely oxidized to p-nitrophenylacetic acid and eliminated in the urine without undergoing either reduction or conjugation.

The simultaneous reduction of a nitro group and the oxidation of some other group within the same molecule is not confined to the nitro aromatic aldehydes, but appears also in the case of nitrobenzol. This substance is first oxidized in the p-position to form p-nitrophenol. The nitro group is then reduced to an amino group yielding p-aminophenol as an end product (72).



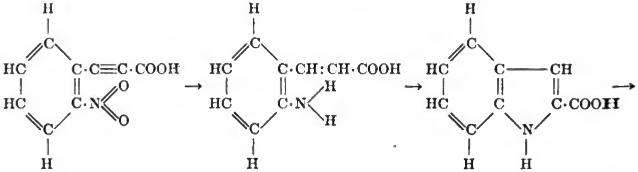
Nitrobenzol. p-Nitrophenol. p-Aminophenol.

If, however, m-nitrophenol (8) is fed to rabbits, only a portion of it is reduced to m-aminophenol. Much more striking, however, is the case of o-nitrophenol which is excreted by rabbits unchanged. Picric acid (2,4,6-trinitrophenol), according to some authors (85) is excreted merely combined with sulphuric acid, while according to others (4), a reduction takes place and the substance appears as picramic acid:

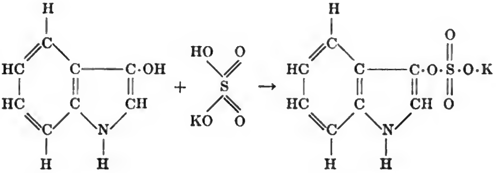


Picric Acid. Picramic Acid.

Perhaps the most remarkable case of reduction ever observed was that noted by Hoppe-Seyler (40), who fed to rabbits o-nitrophenylpropiolic acid and found in the urine a substance identical with urinary indican, i.e., as a compound of indoxyl with either potassium hydrogen sulphate or glycuronic acid. The reaction is apparently that represented below, namely, a reduction of the nitro to an amino group and also a reduction in the side chain with the formation of o-aminoacrylic acid, then a closing of the ring resulting in the formation of indol. This compound thereupon loses carbon dioxide and is oxidized to indoxyl, which is then conjugated with potassium hydrogen sulphate and excreted as indican.

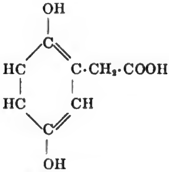


o-Nitrophenyl-propiolic Acid. o-Aminophenyl-acrylic Acid. Indol-2-Carboxylic Acid.



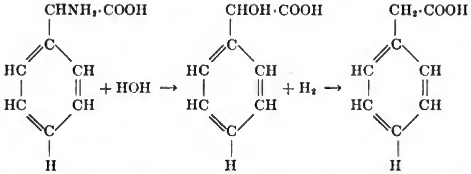
Indoxyl. Indican.

DEAMINATION. Hydrolytic or oxidative deamination of foreign organic molecules takes place in the animal body in much the same way as the reaction pictured for the cleavage of the amino group in the α-amino acids resulting from protein hydrolysis. Abderhalden and Massini (2) have shown that p-aminotyrosine leads to an increase in the homogentisic acid excreted by alkaptonuric patients. This necessitates the hydrolysis of the amino group in the aromatic nucleus with the formation of a p-hydroxy-compound as well as a partial decomposition of the chain.



Homogentisic Acid.

We saw previously that an amino group in the side chain may be removed by hydrolysis with or without subsequent reduction of the chain, for both phenylglyoxylic and phenylacetic acids were found in the urine after feeding phenylglycine (phenylaminoacetic acid).



Phenylglycine. Phenylglyoxylic Acid. Phenylacetic Acid.

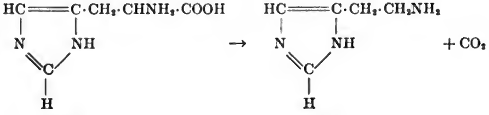
The hydrolysis of the amino group, however, is not confined to those cases in which the amino group occupies the α-position, as is evidenced by the fact that α-β-diaminopropionic acid is converted into the corresponding dihydroxy acid (68):

$$\underset{\alpha\text{-}\beta\text{-Diaminopropionic Acid}}{CH_2NH_2 \cdot CH \cdot NH_2 \cdot COOH} + 2HOH \rightarrow \underset{\alpha\text{-}\beta\text{-Dihydroxypropionic Acid.}}{CH_2OH \cdot CHOH \cdot COOH} + 2NH_3$$

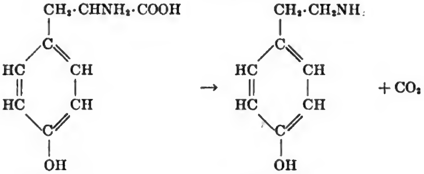
After the action of putrefactive bacteria on proteins and protein decomposition products in the gastro-intestinal tract, the α-amino acids may be decarboxylated, i.e., attacked in such a way that they lose carbon dioxide from the carboxyl (-COOH) group, and thus be converted into the corresponding amine:

$$\underset{\text{Glycocoll.}}{CH_2NH_2 \cdot COOH} \rightarrow \underset{\text{Methylamine.}}{CH_3NH_2} + CO_2$$

Among these amines are substances which exert a decided effect upon blood pressure, such as histamine and tyramine, and are therefore considered as very toxic substances. They may perhaps even be the cause of arterio-sclerosis and subsequently one of the prime factors in producing old age.



Histidine. Histamine.



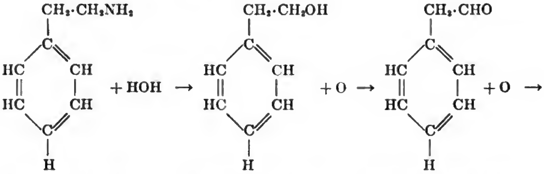
Tyrosine. Tyramine.

The work of Guggenheim and Loeffler (36) has enlightened us somewhat on this point, demonstrating that these substances may be taken in relatively large doses, 0.25 gram or more, without decided physiological effects. The investigators were able to show at the same time that these toxic amines are rapidly detoxicated in the animal body by a simple cleavage of the amino group, perhaps by hydrolysis, yielding the corresponding alcohol, or by reduction yielding the hydrocarbon.

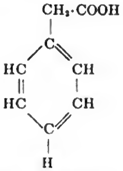
$$\underset{\text{Substituted Amine.}}{R\cdot CH_2\cdot CH_2NH_2 + HOH} \rightarrow \underset{\text{Substituted Alcohol.}}{R\cdot CH_2\cdot CH_2OH + NH_3}$$

$$\underset{\text{Substituted Amine.}}{R\cdot CH_2\cdot CH_2NH_2 + H_2} \rightarrow \underset{\text{Hydrocarbon.}}{R\cdot CH_2\cdot CH_3 + NH_3}$$

In some cases alcohols were isolated after feeding the amines, showing the actual formation of the alcohols as intermediary products, but in most cases the oxidation proceeded further, yielding a corresponding acid. Thus phenylethylamine gives phenylacetic acid, probably passing through the intermediary metabolic forms of phenylethyl alcohol and phenylacetaldehyde, for both of these compounds yield phenylacetic acid when fed by themselves, although phenylethane, $C_6H_5\cdot CH_2\cdot CH_3$, is oxidized to benzoic acid.



Phenylethyl-amine. Phenylethyl-alcohol. Phenylacetaldehyde.



Phenylacetic acid.

Similarly it was found that isovaleric acid was formed from amylamine, p-hydroxyphenylacetic acid from p-hydroxyphenylethylamine (tyramine), and indolacetic acid from indolethylamine. Histamine very likely undergoes a like change but no β-imidazoleacetic acid was identified in the urine.

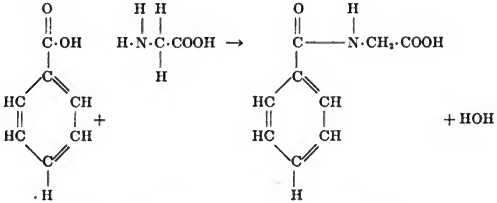
Synthetic reactions. In a few cases foreign organic compounds enter the animal body and are rapidly excreted without undergoing chemical change. These cases, however, are relatively few and it will be found invariably that the substance is non-toxic and quite soluble. If the foreign molecule cannot be destroyed entirely or even partially and still retains after partial demolition some of its toxic properties, the only recourse left to the body is a synthetic type of reaction. This it accomplishes by attaching to the foreign substance an amino or methyl group or by conjugating it with a second molecule such as glycocoll, glutamine or ornithin. In this way not only is toxicity reduced or destroyed, but solubility is simultaneously increased, enabling the body to excrete the product with ease and rapidity. There are cases, too, where a given substance is conjugated partly with one compound and partly with another. This is true, for example, of phenol which, after ingestion, is excreted not only as a sulphuric acid conjugate but also as a glycuronate and even to some extent in the free state. This is perhaps the foundation of the erroneous opinion often expressed in textbooks that conjugation is effected with one of these detoxicating agents, such as glycocoll, for the purpose of protecting the foreign substance against further oxidation, for otherwise the intruder might be excreted uncombined. Upon examining a case of this kind one finds, for example, that after a certain compound is ingested 65 to 75 per cent of it has been excreted in combination, let us say, with glycuronic acid, while the remainder has apparently disappeared. Thereupon the inference is drawn that the 25 to 35 per cent of the substance unaccounted for has been oxidized in the organism and that only by conjugation has the other part been saved from this fate. The truth of the matter is, however, that the body has conjugated this foreign molecule with glycuronic acid only after a futile attempt to oxidize it, and that the 25 to 35 per cent had escaped detection either because of some other channel of elimination or on account of a different form of conjugation. In fact, the recovery of 65 to 75 per cent of many of these substances may be considered "almost quantitative."

Dakin (17) has shown experimentally that phenylpropionic acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$, cinnamic acid, $C_6H_5 \cdot CH \cdot CH \cdot COOH$, and β-phenyl-β-hydroxypropionic acid, $C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$, which are more or less poisonous in themselves, are rendered non-toxic when combined with glycocoll. Phenylpropionic acid when administered to cats in doses so small as 0.8 gram per kilo of body weight caused death in 40 to 60 hours, while phenylpropionylglycocoll in doses as large as 1.5 grams per kilo body weight proved entirely non-toxic.

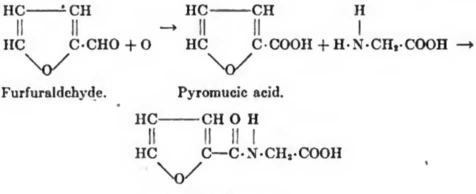
Berczeller (10) has recently suggested an explanation for certain conjugation products from a physico-chemical point of view. He believes that the compounds which are susceptible of conjugation in this way are those which increase the surface tension of the liquid in which they are dissolved, while the conjugation product has less if any effect of this kind. Benzoic acid lowered the surface tension of the solution very decidedly, while hippuric acid produced no such effect. Menthol likewise caused a marked depression of the surface tension. Its detoxication product, menthol-glycuronic acid, however, had much less effect, and the salt of the glycuronic acid compound still less. Phenol exerted the same influence as menthol, while phenol-sulphuric acid and the two oxidation products of phenol, namely, pyrocatechol and hydroquinone, acted like menthol-glycuronic acid and the salt of the latter, respectively. It is interesting to note that glycocoll itself was found to be inactive in this regard. The observations of Berczeller are instructive and no doubt valuable, but serious complications arise when we reflect that a given compound fed to half a dozen different animals is detoxicated in as many different ways.

In general it would seem that a compound incapable of complete oxidation is usually converted into an alcohol or an acid. In the former case there are two main possibilities of detoxication, namely, in the formation of an ethereal sulphate or of a glycuronate. In the case of the acid, conjugation is usually effected wtih one of the amino-acids, such as glycocoll, glutamine or ornithin. Reduction is generally met with in connection with nitro compounds which are thus converted into amines and subsequently often acetylated. As it is impossible to consider the behavior of the individual toxic substances it would seem most logical to take up a short discussion of the several detoxicating agents furnished by the body.

GLYCOCOLL. We have previously seen that aromatic derivatives of the fatty acids containing an odd number of carbon atoms in the side chain are oxidized to benzoic acid while those with an even number of carbons are converted into phenylacetic acid. Benzoic acid is paired with glycocoll by the human being, by the horse, cow, dog, cat, and in fact by every vertebrate thus far used for experimentation with the exception of the fowl (61), (106).



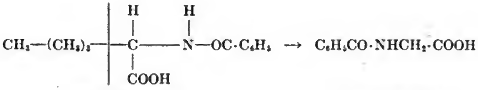
Not only is benzoic acid itself combined in this way with glycocoll but most of the derivates of benzoic acid such as the o- and m-chlor, the m-brom, the o-, m- and p-flour, and the m- and p-nitro compounds as well as the three hydroxy benzoic acids are subjected to a similar fate. Phenylacetic acid is treated in a like manner in the organisms of the dog (87), (88) and cat (89) but not so in that of man or fowl. The same holds for certain derivatives of phenylacetic acid, namely for those compounds resulting from substitutions in the nucleus (30) such as the p-chlor, p-brom and p-iodo compounds. When the substitutions are made in the side chain, however, as in the case of phenylaminoacetic acid, mandelic acid or phenylglycollic acid no conjugation takes place. β-Naphthoic acid, a double ring structure, also combines with glycocoll to some extent (15). A great variety of acids combine in this way with aminoacetic acid. Two rather unusual examples are those of furfural and thiophenealdehyde. As will be seen below, furfural (furfuraldehyde) (49) is first oxidized to pyromucic acid and this in turn combined with glycocoll. A similar reaction is undergone by thiophenealdehyde (14).


Furfuraldehyde. Pyromucic acid.

Pyromucylglycocoll.

The discovery of this means of detoxication about 1825 attributed to Wöhler and noted first in the case of benzoic acid was probably the earliest proof that syntheses of this type could take place in the animal body. For many years thereafter it was thought that the organism merely made use of supplies of glycocoll which it was holding, as it were, in reserve, and that this ready material was simply joined on to benzoic acid by a "dehydration reaction." When it was found, however, that glycocoll could not be stored in the body and that the organism could still provide it, and this in relatively large amounts, even when the glycocoll intake was cut off as in the case of an animal a non-protein diet or during a fast, it then became evident that the body must prepare this compound when required for detoxication purposes. Ringer found that a goat might take 25 grams of benzoic acid per day and excrete it as hippuric acid (83). Magnus-Levy (64) noted that 27.8 per cent of the urinary nitrogen excreted by sheep and rabbits after ingesting benzoic acid might appear in the form of hippuric acid nitrogen; Wiechowski (105) observed that this might run as high as 50 per cent. Ringer expressed the belief that under conditions of benzoic acid feeding the glycocoll is formed from "extra destroyed protein." He is inclined to think, however, that it arises by a specific and peculiar catabolic process rather than from a product of normal intermediary metabolism. Epstein and Bookman (26) conclude from their work on rabbits that benzoic acid exerts a truly toxic effect upon the organism but acting in a certain selective way causes the elimination of much larger amounts of nitrogen than are ordinarily excreted, which nitrogen can be almost entirely accounted for as hippuric acid nitrogen.

It has been suggested by many writers that hippuric acid might arise from a secondary reaction following the primary combination of benzoic acid with one of the long chain amino acids such as leucine, and that this benzoylleucine might then undergo a cleavage resulting in the formation of a glycine rest attached to the benzoyl radical:



$$CH_3\text{—}(CH_2)_2\text{—}\Big|\text{—}\underset{COOH}{\overset{H}{C}}\text{——}\overset{H}{N}\text{—}OC\cdot C_6H_5 \rightarrow C_6H_5CO\cdot NHCH_2\cdot COOH$$

Benzoylleucine. Benzoylglycine.

In order to test this hypothesis, Magnus-Levy (65) benzoylated ten of the amino acids, namely, alanine, serine, valine, leucine, phenylalanine, aspartic acid, glutamic acid and ornithin. He then injected them subcutaneously using a dog as the experimental animal. In each case he recovered the original substance from the urine and this in such large amounts that there was no ground to believe that even a fraction of any of the compounds had been split in the animal organism. The work was to some extent repeated by Shiple and Sherwin (96) who prepared phenylacetyl compounds of alanine, leucine, glutamine, asparagine, glutamic acid, aspartic acid and ornithin and fed them to both men and animals. The results, however, were much the same as those of the former investigator.

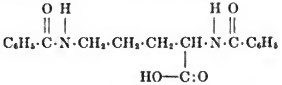
Knoop (54) suggests the possible formation of glycocoll from the oxidation of α-amino-β-hydroxy acids, $R\cdot CHOH\cdot CHNH_2\cdot COOH$, inasmuch as phenylserine, $C_6H_5\cdot CHOH\cdot CHNH_2\cdot COOH$, is incompletely oxidized in the organism and yields hippuric acid. Other experimental evidence, however, is lacking. We must agree, therefore, with Lusk (62) that "these experiments are a further demonstration that in the breaking down of amino acids deamination is the first step, and they leave no conclusion open other than that *glycocoll arises by a synthetic process.*"

McCollum and Hoagland (69) have shown by a series of remarkable experiments that when a pig was reduced to a condition of minimal nitrogen metabolism by a carbohydrate diet (75 cal. per kilo) and at the same time was fed increasingly large doses of benzoic acid, as long as the dose did not exceed 0.2 gram per kilo there was no increase in protein catabolism as evidenced by the constancy in the daily total nitrogen output. Furthermore, there was but little alteration in the various nitrogenous constitutents of the urine with the exception of urea which, very interesting to note, could be reduced from 56 per cent of the total nitrogen to 19 per cent. When amounts of benzoic acid larger than 0.2 gram per kilo were fed there was a decided increase in the output of total nitrogen, much in excess of the amount required for hippuric acid. This shows no more than that 37 per cent of the total nitrogen which ordinarily goes to form urea may under these conditions be side-tracked to form glycocoll. It tells us nothing, however, concerning the mechanism of the reaction. The work has been corroborated by Lewis (60) who fed benzoic acid to human beings maintained on a low protein diet, and by Shiple and Sherwin (98) who reduced a human being to a level of endogenous protein metabolism and were able to show that glycocoll could be formed at the expense of the urea nitrogen without increasing the amount of total nitrogen excreted.

Various attempts have been made to explain the formation of glycocoll by the interaction of ammonia with acetaldehyde, $CH_3 \cdot CHO$, glyoxal, $CHO \cdot CHO$, or glycolaldehyde, $CH_2OH \cdot CHO$, all of which may be considered as decomposition products of carbohydrates. So far, however, the results have been unsatisfactory. Delprat and Whipple in a very recent contribution (22) showed that after injecting benzoic acid into the circulation of fasting dogs there was a definite rise in urea, ammonia, and total nitrogen, provided a certain dosage had not been exceeded. This was certainly to be expected since the demand for glycocoll was extremely acute and the toxicity of the benzoic acid, therefore, increased many times. Moreover, it is impossible to compare the results on fasting animals with those obtained when the animals have been supplied with sufficient food calories in the form of carbohydrates.

From this one hundred years of experimental work we have learned that glycocoll can be built in relatively large amounts by the body even under practically any pathological condition. Moreover, the synthesis proceeds as well during a fast or under abnormal diet as when the ordinary food has been ingested. These results may be far reaching and important since several proteins and particularly the proteins of milk are entirely lacking in this amino acid.

ORNITHIN. Previous work has shown that most of the animals commonly used in experiments supply glycocoll for the detoxication of many of the aromatic acids such as benzoic and phenylacetic acids as well as various of their derivatives. Jaffé (44) however, after feeding benzoic acid to hens was unable to isolate hippuric acid from the excreta, but obtained instead a rather unstable compound which he called ornithuric acid. This substance, later found to consist of ornithin, $CH_2NH_2\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH$, combined with two molecules of benzoic acid has the following structure:



$$C_6H_5\cdot\overset{O}{\overset{\|}{C}}\cdot\overset{H}{\overset{|}{N}}\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{HO-C:O}{\underset{|}{CH}}\cdot\overset{H}{\overset{|}{N}}\cdot\overset{O}{\overset{\|}{C}}\cdot C_6H_5$$

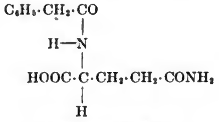
It has since been found that phenylacetic acid (103), as well as p-nitrophenylacetic acid and pyromucic acid (94), (50), combine with ornithin to form compounds analogous to ornithuric acid. In fact no case has yet been observed in which glycocoll is employed in the organism of the bird for conjugation purposes. It is quite reasonable to conclude, therefore, either that birds have no glycocoll at their disposal or, if they have, that they are not able to conjugate it with the various compounds that have been fed. To test this assumption Yoshikawa (107) fed benzoic acid to hens and at the same time added glycocoll to the diet in more than sufficient amounts for combination. No glycocoll compound, however, was found in the excreta. Only the ornithin conjugate appeared, the glycocoll apparently having been burned in the organism. Furthermore, present evidence is lacking both of the formation of glycuronates and sulphates as well as of acetylation. The only synthetic process open to the fowl, therefore, for detoxication of foreign organic compounds, be they acids or alcohols, is so far as we know that accomplished by means of ornithin.

Ornithin is one of the components of the α-amino acid arginine, $H_2N\cdot CNH\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH$. It seems very probable, therefore, that it is formed from arginine when for example birds are fed benzoic acid. Under normal conditions, however, the arginine and consequently the ornithin would be completely destroyed during the processes of metabolism. If this be true, it gives us an important bit of information regarding the intermediary metabolism of arginine, though it also confronts us with a very important question as to the possibilities of the synthesis of this amino acid in the body of the fowl. The only evidence we have on this latter point is that furnished by Thomas (102) who administered benzoic acid to birds maintained on a non-protein diet but was unable to find ornithuric acid in the urine. He found small amounts after adding to the diet edestin which contains large amounts of arginine. It would seem therefore, that arginine and consequently ornithin is not synthesized in the organism of the fowl at the expense of any of the nitrogenous urinary constituents. Since uric acid is the only substance that is present in sufficient amount to furnish the necessary nitrogen, it would be interesting to determine whether under well controlled conditions of experimentation such a transformation is possible. Separation of the urine and feces, however, and the quantitative determination of the urinary constituents and other untold hardships connected with this problem makes such an investigation well-nigh impossible.

GLUTAMINE. Benzoic acid in the organism of various experimental animals as well as that of man is conjugated with glycocoll. It is rather extraordinary, therefore, to say the least, that phenylacetic acid, the homologue of benzoic, should combine in the animal body with glycocoll to form phenaceturic acid, but in the case of the human being with glutamine, $COOH \cdot CHNH_2 \cdot (CH_2)_2 \cdot CONH_2$, to form phenylacetylglutamine, together with some phenylacetylglutamine urea, the latter apparently a simple addition product with urea (101). Still more remarkable, however, is the fact that o-nitrophenylacetic, o-aminophenylacetic, p-nitrophenylacetic, p-aminophenylacetic and p-hydroxyphenylacetic acids pass through the human body absolutely unconjugated. The p-chlor compound is the only one of these derivatives so far examined that probably combines with glutamine. None of these substances combine with glycocoll in the human body, while only the p-chlor (42), p-hydroxy and p-nitro phenylacetic acids have thus far been found to combine with that substance in the organism of any of the lower animals. All the others so far examined pass through uncombined except o-aminophenylacetic acid which is acetylated by the rabbit.

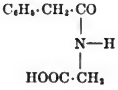
It was at first thought that the phenylacetic molecule was first combined with glutamic acid which product was secondarily changed into glutamine by the addition of the amide group. The feeding of phenylacetylglutamic acid, however, resulted in its excretion without the formation of phenylacetylglutamine. We have here another case of an *amino acid, glutamine,* which it is possible to withdraw intact from the protoplasm of the living cell without its conversion into a secondary or derived product. This furnishes the proof which for a long time was wanting that glutamine rather than glutamic acid must be considered the true form of this -amino acid. Moreover, it explains the genesis of most of the ammonia which always appears when proteins are hydrolyzed in acid media.

Glutamine like glycocoll can be furnished in fairly large amounts for the detoxication of phenylacetic acid (97), and like glycocoll it may be synthesized at the expense of the urea nitrogen (98). A man was placed on a suitable strictly carbohydrate diet and thus reduced to a condition of endogenous protein metabolism. He then ingested on alternate days benzoic and phenylacetic acids. The synthesis of glycocoll and glutamine was accomplished at the expense of the urea nitrogen apparently with great ease in each case. When benzoic and phenylacetic acids were fed together, necessitating, therefore, the simultaneous preparation of glycocoll and glutamine, each aromatic acid was detoxicated and excreted at much the same rate as if it alone had been ingested. It was possible to lower the urea nitrogen from about 60 per cent of the total 24-hour output of nitrogen to about 18 to 20 per cent of the total. During a fraction of a day, however, after 10 grams of phenylacetic acid had been taken, this urea nitrogen was reduced to 12 per cent of the total nitrogen for that period. It was hoped for a time that a solution might be found in these results as to the source of glycocoll in the animal body. For it was just possible that glutamine which is joined with phenylacetic acid and excreted as phenylacetylglutamine by human beings, might in the organisms of the lower animals be an intermediary product which would be subject to further oxidation and appear, for example, as phenylacetylglycocoll. Accordingly phenylacetylglutamine was prepared and fed to dogs, cats and rabbits, but in each case it was excreted without undergoing any transformation into phenaceturic acid. It was also fed to chickens to see what influence it might have on ornithine formation, but again it was excreted without alteration of any kind.



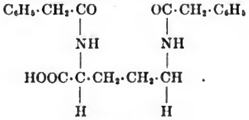
$$\begin{array}{l} C_6H_5\cdot CH_2\cdot CO \\ \qquad\qquad | \\ \qquad H—N \\ \qquad\qquad | \\ HOOC\cdot C\cdot CH_2\cdot CH_2\cdot CONH_2 \\ \qquad\qquad | \\ \qquad\qquad H \end{array}$$

Phenylacetylglutamine.



$$\begin{array}{l} C_6H_5\cdot CH_2\cdot CO \\ \qquad\qquad | \\ \qquad\qquad N—H \\ \qquad\qquad | \\ HOOC\cdot CH_2 \end{array}$$

Phenaceturic acid.

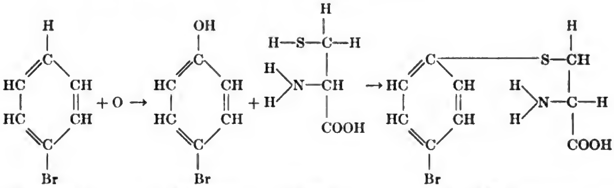


$$\begin{array}{l} C_6H_5\cdot CH_2\cdot CO \qquad OC\cdot CH_2\cdot C_6H_5 \\ \qquad\qquad | \qquad\qquad\quad | \\ \qquad\quad NH \qquad\qquad NH \\ \qquad\qquad | \qquad\qquad\quad | \\ HOOC\cdot C\cdot CH_2\cdot CH_2\cdot CH \\ \qquad\qquad | \qquad\qquad\quad | \\ \qquad\qquad H \qquad\qquad\; H \end{array}$$

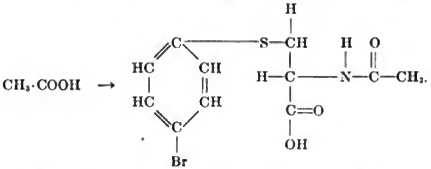
Ornithuric acid.

Similarly phenaceturic acid when fed to human beings failed to undergo synthesis into phenylacetylglutamine. Nor was it altered when fed to chickens, but was simply excreted in each case as the sodium salt of the acid. Likewise ornithuric acid when fed to men and dogs failed to form the phenylacetic acid detoxication products common to these subjects. The results of work with other phenylacetylated amino acids, namely, alanine, leucine, asparagine, aspartic acid and glutamic acid were all negative, for when these compounds were fed to various animals they were always recovered from the urine unchanged. It seems most probable, therefore, that neither benzoylated nor phenylacetylated amino acids are open to alteration of any kind in the animal body.

CYSTEINE. The fourth amino acid to be used by the body for detoxication work is cysteine, $CH_2SH \cdot CHNH_2 \cdot COOH$. It was found both by Jaffé (45) and by Baumann and Preusse (9) that brombenzol, chlorbenzol and iodobenzol, when fed to dogs, is excreted in the urine as a sulphur-containing compound known as a mercapturic acid. Analysis of the substance showed it to be a combination of the benzol halide with an acetylated cysteine molecule. This whole compound, then, more than likely, conjugates with glycuronic acid. The reaction which takes place is probably the following:



Brombenzol. p-bromphenol. Cysteine. p-bromphenylcysteine.



Acetic acid. p-bromphenyl mercapturic acid.

The first step in the reaction is the oxidation of brombenzol in the para position to p-bromphenol, which then combines through its -OH group with the -SH of cysteine to form p-bromphenyl cysteine. This compound is then joined through its amino group with a molecule of acetic acid, resulting in the synthesis of α-aminoacetyl β-thio p-bromphenyl propionic acid, i.e., p-bromphenyl mercapturic acid. Only in the case of dogs has the formation of this compound been established. The partition of sulphur in the urine of human beings and rabbits, however, after feeding the mono-halogen benzol derivatives, seems to indicate the presence of the mercapturic acid, though none has so far been isolated. The chlor- and brom-naphthalenes form a corresponding mercapturic acid to some extent. The p-brom- and p-chlor- phenols, however, strange to say, are excreted in combination with sulphuric acid (58). As cysteine is quite easily oxidized to cystine it is still questionable whether cystin or cysteine is the form in which this sulphur containing amino acid exists in the protein molecule. It is not at all improbable that both forms exist there. If cystine be the primary form, as is generally accepted, then we have the proof in the above mentioned results that cysteine is at any rate a very active intermediary product in body metabolism.

Upon a second consideration of the equations represented above several very interesting questions arise.

1. Is it possible for cysteine to be joined in the original protein molecule through groups other than a similar -SH radical?

2. Is it only in the case of the dog that the mercapturic acid is thus protected against the oxidation of its sulphur, or may this compound be a common intermediary product of metabolism which in other animals is further oxidized and excreted as p-brom-phenol-sulphuric acid?

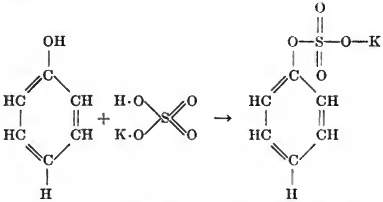
3. What is the significance of the acetyl radical here? Does it hinder or help further oxidation of the cysteine, or is its sole purpose to increase solubility and decrease the toxicity of the compound?

4. Is it possible for the animal body actually to synthesize cysteine for this purpose, or is the organism limited to that which it derives from the food and from decomposition of body tissue?

At the present time there is insufficient experimental evidence to answer any of these questions except the last. Kopfhammer (57) in studying this problem fed dogs with brombenzol after the animals had been reduced to a condition of minimal nitrogen metabolism. He found under these conditions that the dogs excreted no p-bromphenylmercapturic acid. Apparently, therefore, they were unable to form it on a non-protein diet. He again fed brombenzol and injected the dogs subcutaneously with a solution of the sodium salt of cystine at the ratio of 4 grams cystine for each gram of brombenzol fed. Thereupon the animals were able to synthesize bromphenylmercapturic acid in quantities equal to the amount obtained after a heavy protein diet containing much cystine. There is no doubt, therefore, that it is an impossibility for the animal body to build cysteine and subsequently cystine when both sulphur and nitrogen are lacking. It would still be interesting to ascertain whether these amino acids could be built at the expense of the urea nitrogen when sulphur in suitable form is supplied.

ETHEREAL SULPHATES. It was previously stated that when a foreign organic body cannot be destroyed through complete oxidation there is still a tendency to oxidize it as far as possible and either to convert it into an acid that it might be combined with one of the amino acids, or to reduce or oxidize it, as the case may be, into a hydroxy compound or alcohol. In this latter condition, the substance is open to combination with either sulphuric or glycuronic acid. Though there are certain compounds which combine only with sulphuric acid or only with glycuronic acid, still, in general, the substance seems able to combine with either. Apparently the first tendency is to conjugate with the available sulphuric acid. Since this supply, however, is rather limited, combination is next effected with glycuronic acid.

According to our present limited knowledge of the physiological chemistry of sulphur, this element may occur in the urine in three different forms, namely, neutral or reduced sulphur and the two kinds of oxidized sulphur, i.e., inorganic sulphates and ethereal sulphates. Folin (29) has pointed out that the amount of inorganic sulphates in the urine usually runs parallel with the urea output and may therefore, well be considered as resulting from exogenous metabolism, i.e., from the oxidation of the cystine in the food. The excretion of neutral sulphur, however, is much less affected by the amount of protein ingested and therefore, very probably is derived from endogenous metabolism or from the break-down of body tissue, as also from sulphur existing in the bile. This theory is supported by the fact that when cystine is fed it is chiefly excreted as inorganic sulphates while ingested taurine appears mostly as neutral sulphur. The inorganic sulphates, consisting of sodium, ammonium and potassium sulphates, are merely the neutralization products of the sulphuric acid formed by complete oxidation of the cystine or cysteine present in food. The ethereal or conjugated sulphates, finally, are organic sulphates of the nature of an ester, resulting from the union of an alcohol with sulphuric acid. As an example, we have from the work of Baumann and Herter (8) phenol-potassium-sulphate:



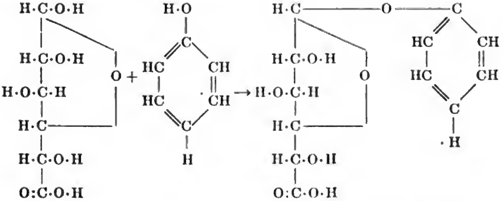
This was the first member of this class of substances to be studied, but since that time more than 200 such compounds have been found. The best known of them are indican (indoxyl-potassium-sulphate), cresol-potassium-sulphate and skatol (skatoxyl-potassium-sulphate), which are the detoxication products of compounds resulting from intestinal putrefaction. The ethereal sulphates in the urine amounting to 0.1 to 0.3 gram per day form roughly 10 per cent of the total sulphate output.

The simplest explanation of the formation of ethereal sulphates is no doubt this, that they arise by a direct combination of the organic base with the inorganic acid salt. Thus when phenol is fed in small quantities it merely joins on to potassium hydrogen sulphate before the second atom of potassium combines with the acid salt to form normal potassium sulphate. That ethereal sulphates are actually formed at the expense of the inorganic sulphates is evidenced by the fact that in cases of phenol and cresol poisoning, where the available supply of sulphuric acid is insufficient for the large amount of poison ingested, the entire output of oxidized sulphur appears in the form of ethereal sulphates to the total exclusion of the inorganic portion. Still, that is not the only explanation of their origin, as can be seen from the following facts. When brombenzol is fed much of it is eliminated in the urine in combination with an acetylated cysteine molecule. The brombenzol, it is believed, is first oxidized to p-bromphenol, which, being an alcohol should combine with sulphuric acid and add to the amount of ethereal sulphates excreted. As a matter of fact, however, combination is effected in this instance through the reduced sulphur group of cysteine. Very probably, therefore, the synthesis of bromphenylmercapturic acid represents a step in the intermediary metabolism of cysteine. Furthermore, it is very likely a type for many similar cases, with this one difference, that in the other instances the resulting compounds appear later on as ethereal sulphates, whereas in the case of p-brom-phenyl-cysteine, for some reason or other, oxidation to a sulphate is, at most, only partial. As a matter of fact, it has been found that after feeding brombenzol there is always an increase of 600 to 1000 per cent in the output of ethereal sulphates and a decided decrease in the amount of inorganic sulphates excreted. This would seem to indicate, therefore, that possibly some of the bromphenylmercapturic acid had been oxidized to p-bromphenylsulphuric acid. There is also an increase in neutral sulphur after feeding brombenzol, but on careful perusal of the data (35), (67), it is easily seen that this is due to the addition of the mercapturic acid sulphur to the normal quota of reduced sulphur and that the latter is not really affected at all.

One would conclude from these experiments either that the ethereal sulphates are directly produced at the expense of the inorganic sulphates by forming esters with sulphuric acid or acid sulphates already at hand, or that they arise from a cysteine conjugate. In the latter case the resulting compound, the analogue of bromphenylmercapturic acid, would undergo oxidation to form the sulphates. The cysteine for this purpose is no doubt mostly of exogenous origin, for mercapturic acid can not be synthesized on a non-protein diet. One might be led to think that ethereal sulphates are also dependent on exogenous cysteine for their formation. Folin, however, has shown that this is by no means the case for he found that the decrease in the ethereal sulphate output on a low protein diet was only relative. The question cannot be fully answered, however, without taking into consideration the sulphur metabolism of the bacteria residing in the intestinal tract.

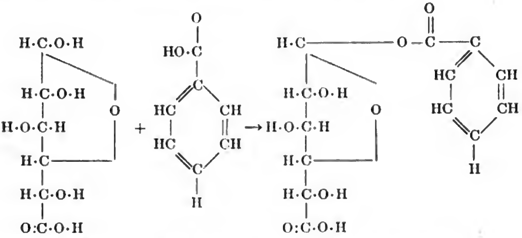
GLYCURONIC ACID. This substance is found in combination with an almost unlimited number of compounds, but when the matter is considered as a whole, it is seen that most of these substances contain a hydroxyl group and may be classed, therefore, as aliphatic or aromatic alcohols. Primary and secondary alcohols are quite easily conjugated with glycuronic acid, for which reason many aldehydes or ketones when fed are excreted in this form of combination after undergoing a reduction to their corresponding alcohols. Although some of the primary and secondary alcohols do not combine with glycuronic acid, practically all the tertiary alcohols appear to do so in the organism of the rabbit.

There are at least two different forms of glycuronic acid combinations and present indications point to a probable third. The first is a *glucoside type* of binding, which often appears as pictured below in cases of phenol poisoning (79) (86).



Glycuronic acid. Phenol. Phenolglycuronic acid (β-Glucoside type.)

The second or ester type of union is found in such combinations as that formed between benzoic and glycuronic acids (47) (64).



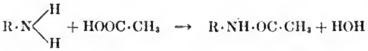
Glycuronic acid. Benzoic acid. Benzoylglycuronate.

To the glucoside type belong the compounds of glycuronic acid with the alcohols and phenols, while the second type is employed in the case of certain toxic organic acids, notably benzoic acid, dimethylaminobenzoic acid, salicylic acid and very likely the halogen phenylmercapturic acids. The ester type of glycuronates reduces Fehling's solution, but the glucoside type will effect no reduction until the compound has been hydrolyzed. The glycuronic acid, which is in itself a strong reducing agent by virtue of its aldehyde group, is thus set free.

There is still much difference of opinion regarding the source of glycuronic acid, as to whether it is formed from glucose and if so whether it is a normal decomposition product of that substance, or whether it originates entirely independently of glucose metabolism. It is rather unreasonable to suppose that this carbohydrate-like compound should be directly synthesized by the body when it could be obtained so simply from glucose. On the other hand, it seems highly improbable that it is a normal catabolic product of glucose since the aldehyde group, which would certainly be the first point of attack when the glucose molecule is subjected to oxidation, is still present in glycuronic acid. Furthermore, the fact that after ingestion of large amounts of glycuronic acid we find gluconic acid, saccharic acid and much oxalic acid in the urine means simply this, that glycuronic acid is oxidized with difficulty, but does not indicate that it is a normal intermediary product of glucose catabolism. Perhaps the most plausible explanation of the origin of glycuronic acid is this, that a union is effected between the aldehyde group of glucose and the foreign molecule, by which the former is protected against further oxidation. The next point of attack then is the primary alcohol group which is converted accordingly into a carboxyl.

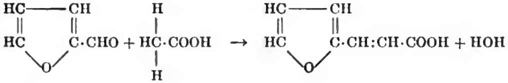
A mass relationship always exists between sulphuric acid and glycuronic acid when there is question of effecting combination with the aromatic substances which are formed in intestinal putrefaction. If the concentration of either of these acids is increased the amount of conjugation with the aromatic poisons will be varied in the direction of the higher concentration.

ACETYLATION. Acetic acid not infrequently, plays an interesting and perhaps important rôle in intermediary metabolism. The usual type of reaction into which it enters is the formation of an acetylamino compound.



$$R\cdot N\begin{matrix} \diagup H \\ \diagdown H \end{matrix} + HOOC\cdot CH_3 \rightarrow R\cdot NH\cdot OC\cdot CH_3 + HOH$$

Under the treatment of reduction, a case of acetylation has been mentioned in which acetylaminobenzoic acid was formed. Still another case was that of the formation of p-bromphenylmercapturic acid. Knoop and Kertess (55) have demonstrated the possibility of acetylation of an γ-amino acid when they recovered γ-phenyl-α-acetylamino-butyric acid after feeding dl-γ-phenyl-α-amino-butyric acid. Likewise dl-phenylaminoacetic acid is excreted to some extent as d-phenyl-acetylaminoacetic acid (78). Perhaps the most striking case of the entrance of acetic acid into one of these reactions was that reported by Jaffé and Cohn (49). After feeding furfural to dogs and rabbits, they found that the methyl group of acetic acid had condensed with the aldehyde group of furfural resulting in the formation of furfuracrylic acid.

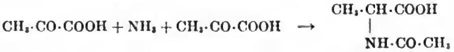


Furfural. Acetic acid. Furfuracrylic acid.

This is exactly analogous to the formation of cinnamic acid by the Perkins reaction from benzaldehyde, acetic anhydride and sodium acetate:

$$C_6H_5{\cdot}CHO + CH_3{\cdot}COOH \rightarrow C_6H_5{\cdot}CH{:}CH{\cdot}COOH$$

It was formerly believed that acetylation was extremely complicated and was brought about only in connection with certain simultaneous processes of oxidation. This view, however, has since been disproved by Ellinger and Hensel (23) who showed that both p-aminobenzaldehyde and p-aminobenzoic acid were acetylated in the organism of the rabbit as completely as p-nitrobenzaldehyde. The reaction occurred, therefore, independently of either oxidation or reduction in the molecule. The more recent work of Miss Hensel (38) on the quantitative studies of acetylation in the animal body tends to confirm the earlier views of Knoop (53) (55) who believed that acetylation is probably produced from pyruvic acid and ammonia. If, for example, two molecules of pyruvic acid interact with one molecule of ammonium carbonate with the formation of carbon dioxide and water, we have formed acetyl-alanine:

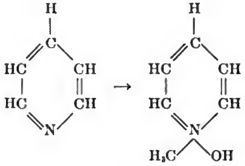


$$CH_3{\cdot}CO{\cdot}COOH + NH_3 + CH_3{\cdot}CO{\cdot}COOH \rightarrow \begin{array}{c} CH_3{\cdot}CH{\cdot}COOH \\ | \\ NH{\cdot}CO{\cdot}CH_3 \end{array}$$

Pyruvic acid. Pyruvic acid. Acetylalanine.

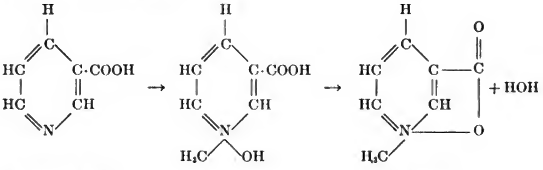
Miss Hensel found that salts of acetic acid actually increased the yield of an acetylated product. Pyruvic acid and aceto-acetic acid also promote the acetylation reaction, a fact which seems to indicate that these two compounds are finally split into acetic acid in normal catabolism.

Methylation. This type of reaction, while perhaps very common in connection with the formation of such compounds as creatine and sarcosine, is rather rare as a detoxication process. One of the first cases of this reaction to be discovered was that observed by His (39), who fed pyridine to a dog and found in the urine a methyl-hydroxy-pyridine compound.



Pyridine. Methyl-hydroxy-pyridine.

It has since been shown that the same reaction is employed also by pigs (104), goats and chickens (41), though not by rabbits (1). A similar example has been reported by Ackermann (3) who found trigonellin in the urine of dogs after feeding nicotinic acid. Here too we have the addition of a methyl and a hydroxyl group to the nitrogen of the pyridin ring followed by a secondary inner anhydride formation by splitting out a molecule of water.



Nicotinic acid. Trigonellin.

Several other examples of methylation might be cited, but in most of the cases the resulting structure is either unknown or so complicated that little can be learned regarding the nature of the reaction. The formation of methylamino compounds in the plant world is very common, but the manner in which they are produced is still a mystery. It has been suggested that the plant accomplishes the reaction by means of formaldehyde and amino groups, but as we have absolutely no proof of the presence of formaldehyde in animal tissues, this explanation is hardly tenable in the case of animal methylation.

Uramino acids. Amino compounds have been isolated in which the amino group either in the aromatic nucleus or on the side chain has formed an additive compound with a $-CONH_2$ rest. This may be considered as the reaction product of the amino group with carbamic acid, $NH_2\cdot COOH$, or with urea, $NH_2\cdot CO\cdot NH_2$, forming respectively water and ammonia as by-products.

$$\underset{\text{Amine.}}{R\cdot NH_2} + \underset{\text{Carbamic acid.}}{HOOC\cdot NH_2} \rightarrow \underset{\text{Uramino compound.}}{R\cdot NH\cdot CO\cdot NH_2 + HOH}$$

$$\underset{\text{Amine.}}{R\cdot NH_2} + \underset{\text{Urea.}}{H_2N\cdot CO\cdot NH_2} \rightarrow \underset{\text{Uramino compound.}}{R\cdot NH\cdot CO\cdot NH_2 + NH_3}$$

In the laboratory this synthesis is effected by treating the amino acid with HCl and potassium isocyanate. It is very probable, therefore, that most of the uramino compounds found in urine are not produced within the organism but are formed during the evaporation of the urine by the interaction of the amino compound with urea. Some of the best known examples of uramino acids are m-uraminobenzoic acid and taurocarbamic acid. The latter, a union of $-CONH_2$ and the amino group of taurine, was thought at one time to be a compound of considerable importance, until Schmidt and Allen (90) showed that taurine actually passes through the body unchanged but on evaporation of the urine combines with urea to form the uramino acid. From time to time uramino acids have been reported, particularly side chain compounds, which crystallized from the unevaporated urine on standing. These very probably were products of synthetic reactions which occurred within the body. In general, one may say that the uramino acids are very likely of much less importance than at first considered.

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THE PHYSIOLOGICAL ACTION OF LIGHT

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The first systematic effort to study the biological effects of light, and its therapeutic uses, was made by Finsen (1) when he founded his Light Institute in Copenhagen in 1896. Much valuable work, both theoretical and practical, has been done there since, with especial success on the therapeutic side, in the treatment of lupus, but the fundamental problem of the mode of action of light on the living cell remains unsolved. Recently, the rapidly accumulating clinical results of light treatment in tuberculosis, rickets, malaria, etc., closely related as they are to the results of x-ray and radium treatment, continually emphasize the importance of this problem and increase its mystery.

It is at first disappointing to find that there is, apparently, in the animal kingdom no effect analogous to the action of light on the chlorophyll system of the green plant, by means of which light energy is stored and oxygen restored to the atmosphere. Although there is a universal conviction that sunlight is healthy, it is certain that people and animals can live a long time in darkness without any noticeably bad results. Blessing (2), who acted as physician to Nansen during his expedition in the Fram, published a report showing that members of the party exhibited no evidence of anemia during the trip. More recently, Grober and Sempell (3) examined horses that had worked for years in coal mines and found no anemia in any case where a satisfactory nutritive condition existed. But, though the physiological effect of sunlight seems at first sight indefinite and of dubious importance, the action of far ultraviolet light on normal tissue, and the action of near ultraviolet and visible light under certain pathological conditions, has been investigated enough to show that there are well-defined effects due to light, closely related to the physiological results of exposure to radium and x-rays. These results are gradually assuming considerable importance in clinical medicine and present theoretically an interesting but illusive problem in physiology. This review will give as briefly as possible the facts that have been experimentally proved so far with a discussion of the theoretical and practical considerations suggested by them.

Effect of light on microörganisms. The most definite result of the early work on the action of light on the living cell was the proof that ultraviolet light exerts a strong lethal action on bacteria. In 1877 Downes and Blunt (4) showed, for the first time, that sunlight retards the growth of bacteria and proved that this was not due to heat since the same result was obtained with tubes cooled in ice.

Marshall Ward (5) in 1893 exposed anthrax bacilli, in gelatine media, to sunlight and, by means of color filters, demonstrated that the effect was due to the violet end of the spectrum. With a solar spectrum projected on the inoculated media the effect was found to begin in the greenish blue.

In 1905 Hertel (6) did the first quantitative piece of work in this subject. He examined the physiological effect of rays of different wavelengths and the same energy, the energy of the light being measured by means of a thermocouple and galvanometer. His results show conclusively that the shorter the wavelength the greater the lethal effect on bacteria and paramoecia (table 1).

TABLE 1

Time for lethal action of light

WAVE LENGTHS μμ	GALVANOMETER DEFLECTIONS			
	10	50	500	
232	60″	10″		Bacteria
280	3′	40″	7″	
334		60″		
383		8′		
440			3 hrs.	
280	3′			Paramoecia
334		14′		
440			3 hrs.	

As the spectrum of sunlight reaches only to 290μμ in the ultraviolet, greater germicidal effects can be obtained by means of artificial sources, such as the quartz mercury arc and bare metallic arcs. In a recent paper by Browning and Russ (7) a tungsten arc was the source of light used. This gives a rich line spectrum extending as far as 210μμ. A gelatine plate was inoculated with microörganisms instead of being sensitized with a silver salt, exposed to the spectrum of the arc through a quartz spectrograph, and incubated at 37°, with the result that bacteria exposed to wavelengths of 296μμ or less were killed. This paper would seem to show that sunlight contains few rays short enough to affect bacteria. But wavelengths somewhat longer than 296μμ are lethal with longer exposure or with exposures at a higher temperature. Thiele and Wolf (8) found that wavelengths greater than 300μμ were harmless to bacteria at a temperature of 14° to 20°C. and lethal at 30° to 40°C. In Ward's experiments the temperature was probably such that the lethal action began in the greenish blue (about 480μμ). No exact experiments have been made as yet giving the upper wavelength limit of the effect at different temperatures.

There is, however, in all the physiological actions of light, a very marked difference between the effect of light from 400 to 300μμ (near ultraviolet) and light from 300 to 100μμ (far ultraviolet). Light greater than 300μμ, being our normal environment, it is obvious that any organism, ordinarily exposed to this light and easily injured by it, would have perished long since. Light less than 300μμ is an unnatural environment and produces in all living cells strong and often very harmful reactions. Since the effect of light is probably due to the photochemical reactions produced when light energy is absorbed it is not surprising to find that the various constituents of protoplasm begin to absorb light strongly in the neighborhood of 300μμ. Much careful and valuable work on the absorption of substances in the ultraviolet has been done by Henri (9). He finds that the abiotic power of light is almost exactly proportional to its extinction coefficient. When the organisms are small the entire protoplasm is affected and the action obeys the laws of simple photochemical reactions. If the organisms are large the effect is a surface one, owing to the small penetration of the ultraviolet light. In the latter case the protoplasm affected may, by a process of diffusion, affect the rest and the process is similar to a complex photochemical reaction taking place at a strongly absorbing surface.

We do not know the exact nature of the photochemical reactions produced in protoplasm by ultraviolet light, although various clues have been suggested. Bovie (10) finds that paramoecia exposed to a sublethal dose of ultraviolet light are so sensitized to heat that they can not stand, even for sixty seconds, a temperature which is an optimum for the controls. He concludes that death from ultraviolet is due to heat coagulation following sensitization by radiation.

Schanz (11) has shown that the effect of ultraviolet light on protein solutions is to make them less soluble, as indicated by their easier precipitation. Chalupecky (12) finds that exposure of egg and serum albumin to ultraviolet light results in an increase in the globulins at the expense of the albumins. Recent experiments in this laboratory show that a dilute solution of egg albumin may be changed so as to react like globulin towards ammonium sulphate after an hour's exposure, in a quartz test tube, to the light of a quartz mercury arc at a distance of 5 cm. Through glass the light has no effect.

There is also some evidence that lipoids are rendered more soluble on exposure to light, thereby possibly explaining the hemolytic action of ultraviolet light on a suspension of washed red corpuscles (13).

Bovie (14) has also shown that a sublethal dose of ultraviolet light inhibits the cell division of paramoecia. He used a magnesium spark through quartz, which gives a strong line at $280\mu\mu$ and obtained the following interesting results. *1*, There is inhibition of cell division with $\frac{1}{25}$ the exposure necessary for cytolysis. *2*, The duration of the inhibition increases with increasing exposure. *3*, The inhibition is followed by an acceleration of cell division which may, for a short exposure, give an increase in the number of radiated individuals over the controls. Shorter wavelengths from a hydrogen tube, filtered through fluorite, gave a strong lethal action but no inhibition of cell division. The reason given for this is that these very short rays are absorbed in 3 to 4 microns of protoplasm and do not reach the nucleus.

Another result of sublethal exposures on microörganisms is reported by M. and Mme. Henri (15). They found it possible, by means of short exposures to ultraviolet light, to transmute anthrax bacilli to cocci, with various intermediate forms. The modified forms, unlike the original bacilli, can obtain their nitrogen from ammonium salts, or the amino acids, and develop best in media containing sugar. They conclude from this that exposure to ultraviolet light destroys the power of the bacilli to secrete proteolytic enzymes while leaving uninjured the ability to form amylolytic enzymes. As, however, the modified form returns to normal on inoculation into animals, there has been no fundamental change produced and the results are of dubious importance. It is, however, interesting to note that they are in direct contradiction to Burge's work (16) in which he killed bacteria by exposure to ultraviolet light, ground them up to extract the enzymes and found the proteolytic enzymes unharmed. The question of the action of light on bacterial enzymes is evidently in need of further investigation.

By means of its lethal action on bacteria, and also on protozoa (17), the sun is of undoubted hygienic value to mankind. But it is probably beneficial in a less indirect way as well, as there is growing evidence to show that light may have a direct action on higher animals.

Effect of light on the eye. Since the eye is the specialized organ of light response it is possible, as suggested by Browne (18), that there may be changes in metabolism due to a reflex stimulation through the eye. In support of this view he cites the chromatic adaptation of certain frogs and fishes through the eye. There is also some experimental evidence that rabbits give off more CO_2 (19) in the light than in the dark, and that when blinded the output of CO_2 is the same. However, this theory is unlikely since most striking physiological effects are produced in higher as well as in lower animals by far ultraviolet light from which the eye must be carefully shielded. The direct effect of light on the eye can be given briefly as follows. Light of wavelength 760–380$\mu\mu$ (the lower limit being variable and depending to some extent on the age of the individual) penetrates to the retina and is perceived as visible light. Light from 380–295$\mu\mu$ is absorbed by the lens and causes it to fluoresce. Light of wavelength shorter than 295$\mu\mu$ is absorbed by the cornea and conjunctiva producing a severe ophthalmia.

The absorption of the eye media for infrared light or heat radiation (40) is equivalent to the absorption of 2.28 cm. of water. Although it is possible that heat may be a contributing cause in glass blower's cataract, there is no evidence of injury to the eye by heat except in eclipse blindness, which is probably due to heat coagulation of the retina (21). Also, visible light probably produces no lasting ill effect on the retina, except in so far as it contributes to the heat effect in eclipse blindness. The discomfort and disturbance of vision caused by the glare of bright light sources is a most complex question which is not well understood at present. It is largely a question of the amount of contrast in the field of view, for with properly diffused light the eye can function comfortably in bright sunlight, which gives an illumination of several thousand foot candles.

Light from 295–385$\mu\mu$, although absorbed by the lens, produces no ill effect as a rule. Most proteins are coagulated by the ultraviolet light which they absorb but the lens protein seems to be resistant in this respect, at any rate for the wavelengths 295–380$\mu\mu$. The light absorbed by the lens causes a strong fluorescence in it and Burge (22) suggests that the fluorescence constitutes a protective reaction, but the nature of the protection is not clear. It may be mentioned, as a matter of interest, that in some nocturnal animals (rats, mice) the lens does not fluoresce to waves of this length. Burge (22) has shown by a series of ingenious experiments that, in the presence of certain salts, lens proteins coagulate with light longer than 300$\mu\mu$ and he supposes that a combination of nutritive disturbances and strong light may account for the development of cataract. With this possible exception, however, the light absorbed by the lens is harmless.

Far ultraviolet rays are all absorbed by the conjunctiva and cornea. Light of this wavelength (less than $295\mu\mu$) results in a severe conjunctivitis and, with a long exposure, corneal ulcers are formed. Artificial lights, if glass covered, are therefore harmless and sunlight rarely contains enough far ultraviolet to produce injury. However the ultraviolet reflected from large areas of water and from snowfields may result in the temporary injury known as snowblindness. Artificial illuminants, which emit a large amount of radiation of wavelength less than $295\mu\mu$, such as the quartz mercury arc and bare metallic arcs, are known to be extremely injurious and the eyes should be carefully protected from them.

TABLE 2

Transmission of light by the skin

	WAVE LENGTH ($\mu\mu$)							
	436	405	366	354	313	302	297	289
Per cent transmitted by skin 0.1 mm. thick	59	55	49	42	30	8	2	0.01
Per cent transmitted by skin 1.0 mm. thick	0.5	0.3	0.08	0.02				

TABLE 3

Thickness of protoplasm absorbing 90 per cent of light

Thickness of protoplasm (μ)	79	58	18	9	6	3.8
Wavelength ($\mu\mu$)	240	238	231	226	219	214

Effect on the skin. It is difficult to give exact figures as to the extent to which light of different wavelengths penetrates the skin, although a number of papers have been published on the subject. The following quantitative results have been given by Hasselbalch (23) (table 2).

Henri (9) gives the thickness of protoplasm which absorbs 90 per cent of ultraviolet light of different wavelengths (table 3).

Glitscher (24) gives a curve combining his results with those of Hasselbalch, which is shown in figure 1. Roughly one can say that light less than $300\mu\mu$ is absorbed by the epidermis in a layer 0.1 mm.

thick. The shorter the wavelengths the smaller the layer that will completely absorb them. Blood serum absorbs everything below $300\mu\mu$ (25). Normal blood absorbs all wavelengths less than $450\mu\mu$ and has two absorption bands at 540 and $575\mu\mu$. Bloodless tissue is found to transmit some light throughout the visible spectrum.

As a result of the absorption of ultraviolet light in the epidermis, the familiar inflammatory reaction known as sunburn is produced. The end result of this inflammation is the deposition of the pigment melanin in the basal cells of the epidermis. Melanin is assumed to be the end product of the oxidation of tyrosin or of one of the related cyclic compounds, probably through the agency of an oxidizing ferment such as tyrosinase. It is not known how light initiates this reaction and there are many theories as to the rôle that pigmentation plays in human physiology. There is a widespread conviction, not however based on very accurate observations, that people who do not tan respond badly to heliotherapy. Rollier (26), whose alpine sanitarium for the light treatment of tuberculosis has had such great success, is convinced that

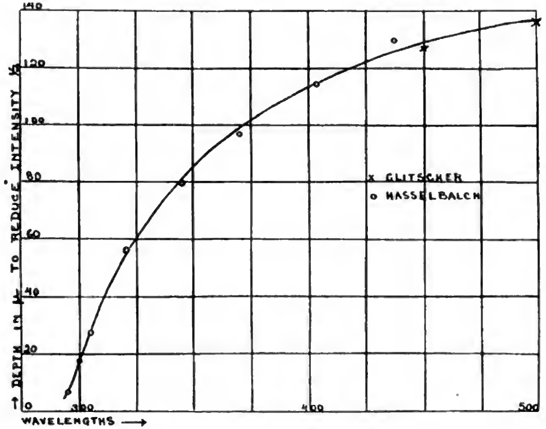

Fig. 1. Absorption of light by the skin.

the beneficial action of sunlight is due to the pigmentation produced. He has a theory that pigment, by fluorescent action, transforms short lethal rays to long non-lethal rays of a greater penetration, thereby doing away with any danger from far ultraviolet light and increasing the depth of light absorption. There is no experimental basis for this theory, and on examining the human skin under Wood's invisible monochromatic light (wavelength 366$\mu\mu$), which produces the most beautiful fluorescence in all substances possessing that property, pigmented areas, such as freckles, are found to be entirely non-fluorescent. It is possible that the chemical change taking place in the epidermal cells, resulting in the formation of pigment, acts in some way as a stimulus to metabolism. The question cannot be definitely discussed since little is known about these chemical changes and one can not definitely say just which rays are responsible for the formation of pigment. It is formed, we know, by the ultraviolet radiation in sunlight so that wavelengths greater than 290$\mu\mu$ are effective and experiments by de Laroquette (27) show that sunlight through glass, which cuts off wavelengths less that 330$\mu\mu$, produces only a slight degree of pigmentation. The most effective rays in sunlight seem, therefore, to be between 290 and 330$\mu\mu$. Jungling (28), although he shows that solar erythema is only produced by rays less than 330$\mu\mu$, the effect increasing rapidly with decreasing wavelength, finds that pigment can be produced by rays greater than 330$\mu\mu$ and that rays less than 290$\mu\mu$, while producing a violent inflammation, do not result in heavy pigmentation. Apparently these very short wavelengths are absorbed before they reach the basal cells of the epidermis and are therefore less effective than the ones from 290 to 330$\mu\mu$. Owing to the difficulty of filtering ultraviolet light so as to obtain only rays less than 290$\mu\mu$, it is not known how short a wavelength is able to produce pigmentation.

It is undoubtedly true that one function of pigment is to act as a protection to the underlying tissues against too much radiation. But at the same time it insures a maximum absorption of radiant energy in the basal cells of the epidermis and may therefore result in an increased light reaction at that point. Ultraviolet light of wavelength shorter than 290$\mu\mu$ would not reach these basal cells except where the epidermis is less than 0.1 mm. thick, so that pigment affords no protection against radiation shorter than that found in sunlight. Any speculation on the rôle played by pigmentation suggests a comparison between the response of white people and negroes to heliotherapy. As nearly all of the published work on heliotherapy is foreign, and most of it German, this comparison has not yet been made, so far as the writer knows.

Light as a stimulus to plain muscle. Adler (29) has experimented with the effect of light on surviving organs and finds that ultraviolet radiation can act as a stimulus to the stomach and intestines of the frog, and to the uterus of the rabbit and guinea pig. Visible light has no effect unless the organs are sensitized with some dye such as eosin or hematoporphyrin (see section on photodynamic sensitization), in which case it acts as a stimulus also.

The effect of light on the blood. Numerous investigators have studied the effect of light on the blood and their results, although usually complicated by the effects of heat and altitude, agree fairly well. The effect of sunlight on the erythrocyte count is not very marked. For short exposures there is no response and some observers have reported negative results when the exposures extended over a considerable length of time (30). However, enough observers have obtained positive results to make one believe that there is a decrease in the red count and the percentage of hemoglobin in the dark and an increase in the light, after long exposures (31), (32), (33). These changes are not permanent and the blood count later returns to normal (2), (3).

On the contrary, the white blood cells, especially the lymphocytes, respond to short exposures of any radiation (sunlight, ultraviolet light, x-ray, heat). Murphy (34) has shown that a 5-minute exposure to dry heat at 55° to 65°C. will produce a rise in lymphocytes of 100 to 200 per cent over the normal count. Russ (35), Taylor (36) and others have shown that on exposure to x-rays the lymphocytes fall rapidly. The response to light is less striking but certain definite results have been found and all the published results agree in the conclusion that ultraviolet light stimulates a lymphocytosis in men and animals (37), (38), (39), (40). In animals it is found (40) that this ultraviolet lymphocytosis is due entirely to rays shorter than $330\mu\mu$. Ultraviolet light from 330 to $390\mu\mu$ diminishes the lymphocyte count slightly but light from a metallic arc very rich in wavelengths shorter than $330\mu\mu$ produces a marked lymphocytosis reaching its maximum in about five days. The production of lymphocytosis, as well as the formation of pigment by ultraviolet light may well be an important factor in heliotherapy. The means by which this lymphocytosis is produced is as yet a mystery. Bunting and Huston (41) have recently published experiments showing that more lymphocytes enter the blood stream from the thoracic duct during twenty-four hours than are present in the blood at any time. The excess of cells migrates from the blood vessels into the mucous membranes, chiefly the gastro-intestinal tract. A lymphocytosis might result either from an increased rate of production or from a decreased rate of destruction, probably from the former. Presumably the lymphocyte-forming organs are stimulated to greater activity by some photochemical change produced by ultraviolet light. Blood radiated outside the body, and then introduced into the blood stream, has no effect on the lymphocyte count (40), so that whatever the photochemical reaction may be it probably takes place in the surface tissues through which the blood circulates, or in the walls of the capillaries themselves.

Most interesting effects of ultraviolet light on the internal organs have been reported by Levy (42) and Gassul (43). Mice were radiated with ultraviolet light for periods ranging from 10 minutes to 56 hours. The animals were then killed and the internal organs examined both macroscopically and microscopically. The spleen, lungs, and liver were found engorged with blood. The spleen was most affected, having increased to two or three times its normal size, partly due to a very great hyperemia, partly to the development of large masses of connective tissue around the follicles. It is apparent then that the absorption of ultraviolet light in the surface of the body to a depth of about 0.1 mm. results not only in an immediate or direct effect on the skin, as indicated by its inflammation and pigmentation, but also in an indirect or distant effect on such organs as the spleen and lungs, which can be explained only by a cutaneous reflex of some sort, or, more probably, by the assumption that some photochemical product, formed at the surface, is conveyed to the internal organs by the blood. The congestion of the lungs was such that the red cells had passed through the capillary walls into the alveoli. This may explain why heliotherapy in lung tuberculosis is frequently followed by hemorrhage.

Ultraviolet light has a hemolytic action on a suspension of washed red corpuscles, outside the body. In the presence of serum this effect does not take place. The protective action of serum, according to Schmidt and Norman (44), is due to certain amino acids, especially tyrosin and tryptophan, owing to the fact that these substances absorb the far ultraviolet. There is no evidence of this action in vivo, probably owing to the protection afforded by the skin and serum.

Effect on metabolism. Light exerts some influence on body metabolism, as is shown by a number of results indicating a change in amount of CO_2 expired, a change in rate and depth of respiration, and an increased rate of growth in the light compared to the dark. However, no results definite enough to review at any length are reported in this field. Since ultraviolet light is a powerful oxidizing and reducing agent it seems strange that it does not produce more marked changes in metabolism than it does. The small penetration of the chemically active rays is of course our protection against them. As far as the change in CO_2 output is concerned, it is visible light and ultraviolet light greater than $330\mu\mu$ which is responsible for the effects reported so far, as the animals experimented on were always exposed to light under glass. None of these experiments are recent (19), (45), (46), but they suggest that it would be worth while to study the basal metabolism of human beings in the light and the dark, and in light of different wavelengths. Very recently experimental work on rickets (66), (67) has given evidence that light is concerned in the phosphorus and calcium metabolism of the body. The results of this work are discussed further under the section on heliotherapy.

Photodynamic sensitization. Although ultraviolet rays, particularly those below $300\mu\mu$, produce many reactions in living cells, visible light is apparently without any effect except in producing vision, heat, and possibly some change in metabolism. It is however possible to sensitize living cells, just as one sensitizes a photographic plate, and produce an abnormal condition in which visible light is as active as ultraviolet. This phenomenon has been called photodynamic sensitization. It was discovered accidentally by Raab (47). Under the direction of von Tappeiner he was stuying the toxic action of acridin on paramoecia and the discordant results in determining the minimum fatal dose led to the discovery that acridin is lethal only in the light. With a strength of 1:20000, paramoecia are killed in 6 minutes in direct sunlight, 1 hour in diffuse daylight, and are unharmed in the dark. After this fact was discovered the subject was extensively investigated by von Tappeiner, Jodlbauer, and their co-workers (48) and many substances were found to act as sensitizers, fluorescein and its derivatives being especially potent. In vitro, a surprising number of interesting results was obtained and it was found possible to sensitize, to the action of visible light, bacteria, protozoa, red blood corpuscles, enzymes, ferments, the various substances concerned in immunity, and certain well-defined chemical substances, such as the combination of mercuric chloride and ammonium oxalate.

Von Tappeiner laid great stress on the fact that most of the substances which act as sensitizers are fluorescent. Photodynamic action however is not proportional to the degree of fluorescence and, although only that region of the spectrum is effective which is absorbed by the fluorescent substances (bluish green for eosin, violet for quinine), it is not always the region that gives the strongest fluorescence that gives the greatest photodynamic sensitization. Also a number of non-fluorescent substances act as sensitizers of photographic plates. Von Tappeiner considers this a different phenomenon, but as Sellards (49) says, in his interesting review of the subject, it is hard to see why one should make any distinction between the oxidation of substances such as KI or metallic silver by eosin and light, and the acceleration of a photographic plate by chlorophyll.

The light emitted by the fluorescent body is in itself ineffective. It is necessary that the sensitizer be in contact with the material on which it acts. Von Tappeiner probably laid a great deal too much stress on the importance of fluorescence in photodynamic action. It is the usual accompaniment but not the fundamental cause of the sensitization, a sort of outward and visible sign, so to speak, of an inner activity in response to light. Fluorescence, in fact, is a more general property than is usually supposed. In a dark room, under Wood's invisible light (quartz mercury arc through a filter transmitting only wavelength $366\mu\mu$), nearly all substances show some degree of fluorescence.

There is no evidence that the sensitizer is permanently altered by exposure to the light. If eosin is exposed to light and then added, in the dark, to a solution containing bacteria, or washed red blood corpuscles, it is as harmless as if it had never been exposed. The presence of oxygen is necessary for most of the effects produced by visible light and the only light which is effective is light of wavelengths corresponding to the absorption band of the sensitizer.

The sensitizing reactions are specific. Although eosin seems to be a sensitizer for all cells and chemical substances, this is not invariably true of other dyes. For instance, methylene blue acts as a sensitizer to katalase but not to peroxidase, and the relative sensitizing action of various dyes is different towards different substances. The specificity of the phenomenon is especially striking when the experiments are tried in vivo. Although a great many substances sensitize in vitro, only eosin, chlorophyll and certain derivatives of hemoglobin have so far been found effective in vivo and the only markedly effective sensitizer for higher animals is hematoporphyrin. This substance is derived from hematin by removing the iron. It has the formula $C_{34}H_{38}O_6N_4$, but is usually obtained and used in its crystalline compound with hydrochloric acid. In vitro it is a less effective sensitizer than the members of the fluorescein group, but in vivo it produces the most marked effects.

Hausmann (50) injected white mice with it and found 0.01 gram harmless in the dark while 0.002 gram will bring on acute symptoms in the light. There is a marked but temporary hyperemia of the ears, nose and tail, and after a period of great activity the animal becomes quiet, shows dyspnea and dies in one to three hours. If exposed to a less intense light or if exposure to an intense light takes place some time after injection, the mouse develops a subacute form and dies in one to two days. With still less light, or a longer interval between injection and exposure, the animals develop a chronic form and remain sick and light-sensitive for months. The blood of the hematoporphyrin mouse is not hemolysed and it is probable, from the length of time that an animal remains sensitized, that hematoporphyrin forms a stable photosensitive compound with some element of the skin tissue which is only slowly broken down and eliminated.

As mentioned above, Adler (29) found that visible light acts as a stimulus to plain muscle in various organs, when they are sensitized with different dyes, and Amsler and Pick (50) have made numerous experiments with light on the surviving frog's heart. Neither eosin, nor hematoporphyrin, nor visible light alone affects the surviving heart injuriously but if a small amount of sensitizer is added to the perfusing solution, and the heart then radiated with visible light, the illumination produces a disturbance in the atrio-ventricular transmission of the impulse, resulting in a sort of heart block, in which the nervous rather than the muscular parts of the organ are affected.

There are a few instances of spontaneous sensitization in which the skin is abnormally sensitive to light. Buckwheat poisoning, among animals, is the best instance and it has been suggested that the skin lesions in pellagra indicate a light-sensitive condition in man. Meyer-Betz (52), with more devotion to science than to his own welfare, tried the effect of hematoporphyrin on himself, by injecting 200 mgm. into his own blood. Subsequent exposure to light produced most distressing symptoms similar to those seen in the mouse and he remained light-sensitive for a long time. In the light-sensitive condition, known as hydroa vacciniformis, hematoporphyrin is found in the urine and it has been suggested that it is an example of naturally occurring hematoporphyrin sensitization, since the symptoms bear some resemblance to Meyer-Betz' reactions. However, Sellards (49) after working at the problem from many different angles came to the conclusion that, on the whole, sensitization plays but little part in the etiology or therapy of the diseases of man. Bile pigments are photodynamic in vitro, but apparently the body can be overwhelmed with highly hemolytic pigments without suffering serious unjury. There is, perhaps, some mechanism protecting the body against the hemolytic action of bile pigments which is not so efficient toward hematoporphyrin.

As has been explained above, the usual meaning attached to photodynamic sensitization is the production by visible light, plus a sensitizer, of the same reaction that ordinarily takes place under ultraviolet light. It has however been shown by Howell (paper not yet published) that there is another type of sensitization, in which dyes plus visible light produce an effect opposite to that produced by ultraviolet light alone. Fibrinogen, freshly prepared, coagulates spontaneously under ultraviolet light. If exposed through glass there is no spontaneous precipitation, but it coagulates on heating to 60°C. or on the addition of thrombin. On adding eosin, or hematoporphyrin, and exposing to either ultraviolet or visible light, the fibrinogen is not only desensitized to heat, so that is fails to coagulate on heating to 90°C., but also fails to to react with thrombin. Whether the action of hematoporphyrin on higher animals belongs to this type of sensitization, or to that usually observed, is not at present known.

Heliotherapy. The results of heliotherapy are entirely empirical and not at all based on the experimental facts of light action as outlined above. They suggest, however, many valuable lines along which experimental work should be done in the future.

1. Skin diseases. Much use has been made of ultraviolet light in various diseases of the skin with favorable results in many conditions, but the most striking positive result is the work of Finsen (1) in the treatment of lupus vulgaris. By means of concentrated sunlight or arc light, from which the heat was eliminated as much as possible, he was able to cure lupus in the great majority of cases. The light destroys the diseased tissue and promotes the growth of the healthy tissue. The treatment is a long one but on the whole safer and more successful than treatment by x-rays. Eczema and acne are sometimes cured by light, and birthmarks may be improved, but the same or better results can be obtained with x-rays. One advantage of ultraviolet over x-ray treatment is that it is much safer. Even when the skin is severely burned no bad after-results are noticed.

2. Tuberculosis. In addition to its effect on tubercular skin infections, light has a markedly beneficial action on most of the other forms of tuberculosis, particularly the surgical types. In 1903 Rollier (26) founded a sanatorium at Leysin for the treatment of tuberculosis by heliotherapy and his results, especially with surgical tuberculosis, are so promising that the method is gradually being adopted elsewhere. Rollier's treatment consists of sunbaths, beginning with a short exposure of the feet, and gradually increasing the area exposed and the length of exposure until the whole body, except the head, is exposed for several hours a day. This light treatment, combined with outdoor life and a high altitude results in a remarkably large percentage of cures. The following statistics, quoted from Gassul's monograph (53) cover 1129 cases, 652 of which were adults (table 4).

The most striking result is in bone and joint tuberculosis. Exposure to sunlight increases the rate of disintegration of cells damaged beyond repair, while stimulating the activity of normal cells, and acting also as a stimulus to recalcification. X-ray photographs, after the light treatment, give striking evidence of the effect upon bone formation. According to Rollier, phalanges that have entirely disappeared may be so completely recalcified as to be indistinguishable, in radiographs, from normal tissue, and adults seem to be as easily affected as children. This means that, for early cases at least, the disease can be checked, and motion preserved in the affected joint, the gradual establishment of motion going hand in hand with the healing process. There is also a marked reaction in sinuses and ulcers. Under light treatment there is a profuse discharge followed by sloughing, formation of healthy granulations and healing. In tubercular lymph glands there is a gradual reduction in size and in broken-down glands the contents are frequently absorbed. There is absorption of effusions in the joints, and in the peritoneal and pleural cavities, which is especially noticeable in peritonitis. A recent paper by Hyde and Grasso (54) gives details of

TABLE 4

Results of light treatment in surgical tuberculosis (Rollier)

	CURED	IMPROVED
	per cent	*per cent*
Skin tuberculosis	81.25	18.75
Bone and joint	75.98	7.80
Glandular	89.80	5.20
Peritonitis	80.30	8.20
Genito-urinary	77.80	22.20
Kidney	52.94	33.00

Total mortality 0.9 per cent.

Rollier's method and the results of its application at Perrysburg, N. Y. Their results, though slightly less favorable throughout, are roughly similar to those of Rollier, given in table 4, and indicate that heliotherapy can be successfully used for surgical tuberculosis even at low altitudes. They report the use of the quartz mercury arc on dark days but say that it is inferior to sunlight. There are no conclusive results on the exclusive use of the mercury arc so that it is impossible to say how far it may be used to replace sunlight. There is some evidence that mercury arc treatment is useful in intestinal tuberculosis but in lung tuberculosis both sunlight and artificial light baths are apt to lead to hemorrhages, possibly on account of the congestion of the lungs, which is known to follow exposure to ultraviolet light in the lower animals (42), (43).

How sunlight acts upon tuberculosis is only conjecture at best. Rollier insists on the fact that the benefit is always proportional to the degree of pigmentation. Without knowing accurately which wavelengths are responsible for pigmentation and which are most beneficial in the treatment of tuberculosis, any connection between the two effects is largely guesswork. It may be that the chemical changes resulting in pigmentation give a stimulus to the entire body metabolism. It may be that after it is formed the pigment acts as a sensitizer to light. On the other hand, the production of a lymphocytosis may be the important result. It is difficult to get a clear picture of the blood changes due to heliotherapy, as the blood picture in different types of tuberculosis is different to begin with, and the effect of altitude usually complicates the results. Rollier (26) states that, with heavy pigmentation and good prognosis, lymphocytosis takes place. Murphy (55) has found that mice with a high lymphocyte count are resistant to the inoculation of tubercle bacilli, so that the blood changes may well be the important ones. If this is correct, wavelengths less than $330\mu\mu$ should be the most effective in producing good results.

Gassul (53) in a recent monograph has given a summary of the results of treatment of tuberculosis with radiations of every type (light, x-ray, radium). Figure 2 is taken from his paper and shows most graphically the high percentage of cures possible by radiation treatment.

3. Wounds. In connection with the effect of light on tuberculosis it is interesting to note that a number of papers have been published on the use of ultraviolet light in the treatment of wounds (see review by Breiger (56)). The benefit may be partly due to the bactericidal action of the short wavelengths but there is also a marked effect similar to the action on tubercular lesions. The diseased tissue is destroyed and the growth of healthy granulations stimulated.

4. Rickets. In tuberculosis of the bones and joints light is found to act as a recalcifying agent and this property makes it also a beneficial agent in rickets. A number of papers have appeared in foreign journals on this subject (57), (58), claiming that under light treatment, with the quartz mercury arc, the recalcification of bones proceeds at an accelerated rate, and that in spasmophilic rickets symptoms of tetany disappear. These experiments were made on human subjects and all the evidence has been furnished by radiographs. The study of this effect has recently been taken up in this country in a more systematic way (59), (60), (61), (62). It seems now well-established, by experiment, that rats, fed on a diet which is known to produce rickets, fail to develop the disease if they are exposed daily, for a short time, to sunlight or to the quartz mercury arc. A study of the chemical changes in the blood under sunlight treatment for rickets by Hess and Gutman (61), showed

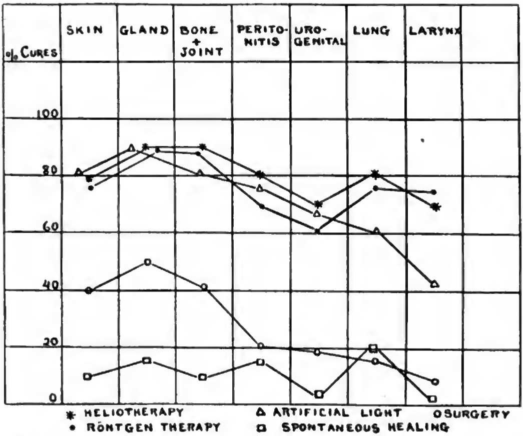

Fig. 2. Percentage of cures in surgical tuberculosis under different methods of treatment.

that the inorganic phosphorus of the serum, which is reduced in children suffering from rickets (63) comes back to normal under sunlight treatment, or on the administration of cod liver oil. These two agents seem to stimulate the deposition of inorganic salts and particularly affect the phosphorus metabolism of the body. These results are extremely interesting from a clinical standpoint and also give the first definite experimental evidence of metabolic change in the animal body brought about by sunlight.

5. Malaria. There is some evidence for believing that, in cases of chronic malaria, relapses may be induced by ultraviolet light (64), (65), (66). This is interesting in connection with the experiments by Levy (42) and Gassul (43) on the effect of ultraviolet light on the spleen. Work in this direction, however, has not progressed far enough to make any definite statements possible.

It has often been suggested that quinine owes its curative properties to its photodynamic action. This theory has never had any facts to substantiate it and has been thoroughly disproved by Sellards (49).

Ecology. There seems to be no doubt that, with extended knowledge as a result of more exact experiments, heliotherapy will be able to produce definite and valuable results. So far the experiments have seldom been with monochromatic light and little distinction has been made between the ultraviolet in sunlight and in artificial sources. As information on the light emission of different sources and the absorption of various materials is scattered and hard to find when needed, tables giving some of these facts have been put at the end of this paper (tables 6 and 7).

The study of the extent and intensity of the ultraviolet radiation in sunlight is of particular interest on account of its application both to heliotherapy and to ecology. In connection with his studies on the effect of the Philippine climate on man, Freer (67) determined the limit of the solar spectrum in the ultraviolet and found it to be $291\mu\mu$, which is identical with the limit as found at all latitudes and altitudes. Miethe and Lehman (68) made measurements at Assuan, Berlin, Zermatt, Görnergrat and Monte Rosa and found the limit of the spectrum always the same, 291.21 to $291.55\mu\mu$. This limit, being fixed by ozone absorption in the outer atmosphere, is independent of any condition on the earth. However, the intensity of distribution in the solar spectrum, particularly in the ultraviolet end, is a different matter. Dorno (69), in a recent book, has summarized the findings on this subject and given the results of his own valuable work at Davos. He studied the daily and yearly variations of the ultraviolet and blue in sunlight by means of photo-electric cells. Brightness determinations were made with a photometer, and bolometric and pyrheliometric determinations were made of solar heat. Most accurate heat measurements have been made by Abbot (70) and others but Dorno's work on the ultraviolet variation is unique. Figure 3 from his book shows the extraordinary variation in the intensity of ultraviolet radiation throughout the day, and also the great difference between a day in summer and one in winter. Figure 4 also shows that the midday variation throughout the year is markedly greater for ultraviolet than for heat and visible light. Latitude and altitude must also make a great difference in ultraviolet intensity because with increase in altitude the transparency of the atmosphere increases faster for ultraviolet than for visible light. Since the ultraviolet light undoubtedly exerts an influence on the human organism, further studies in this field should yield most important ecological results.

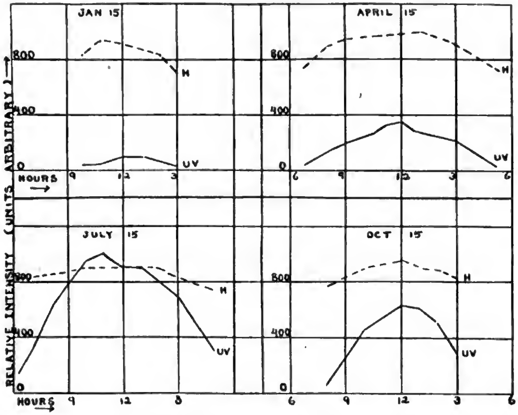

Fig. 3. Variation in intensity of solar heat, *H*, and ultraviolet solar radiation, *UV*, throughout the day, in different months. Experiments at Davos, Switzerland.

Freer (67), Chamberlain (71) and others have carefully studied the effect of a tropical climate on the condition of soldiers in the Philippines, especially in reference to the effect on blondes. They found that all soldiers, irrespective of their coloring, reacted in the same way to life in the tropics. They showed loss in weight, higher pulse rate and rate of respiration, and a lower blood pressure. They had a high red count and low color index, and the differential white count showed a high percentage of lymphocytes and a low percentage of polynuclears (72).

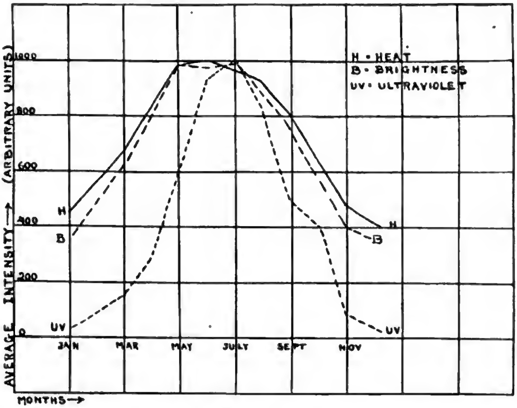

Fig. 4. Average midday intensity of solar heat, *H*, ultraviolet radiation, *UV*, and brightness, *B*, throughout the year at Davos, Switzerland.

The same changes in pulse, respiration, and blood pressure can be brought about by moist heat (73), and it is quite possible that the heat may also be responsible for the blood changes. On the whole, the work in the Philippines showed that the deleterious effects of the tropics are due to heat and not to light. Now in tuberculosis and rickets, and possibly in other diseases, light has a curative action, and the beneficial effects are probably located in the ultraviolet region less than 330$\mu\mu$. It seems then conceivable that the benefit we obtain from outdoor life may be due, in part at least, to the ultraviolet of the sunlight. Indoor light is cut short at 330$\mu\mu$ by the absorption of glass.

A theory of light action. With our increasing knowledge of atomic structure, and the importance of the configuration of electrons in an atom in determining its physical and chemical properties, we are beginning to interpret chemical as well as physical reactions in terms of electronic behavior. In a general way it is safe to believe that the physiological effects of light have their origin in the photochemical reactions produced when light is absorbed. With simpler chemical substances light is seen to act as a powerful oxidizing and reducing agent, so we suppose that with the more complex chemical compounds in the living cell the same is true. Also, it is safe to suppose that all photochemical reactions are initiated by a change in configuration and velocity of the electrons of the substance absorbing the light. Light energy, absorbed by a body, may increase the molecular motion, thereby producing a rise in temperature. If the incident light has small enough wavelengths to produce vibrations in the electrons, instead of in the molecules and atoms, the absorption of light may result in the escape of electrons from the atoms, with a consequent change in valency, resulting in chemical action. When light of very short wavelengths (less than 300$\mu\mu$) falls on many substances, notably metals, where most of the energy is absorbed in the surface layer of atoms, the electrons absorbing this energy have their own energy increased to such an extent that they are shot off from the surface of the metal, leaving it positively charged. This phenomenon, discovered by Hallwachs in 1888, is called the photoelectric effect.

There is *1*, the normal photo-electric effect, shown by a great number of substances, in which the number of electrons shot off increases with decreasing wavelength of the incident light; and *2*, the selective effect, shown only by the alkali metals, in which a great number of electrons are shot out by light of a particular wavelength in a particular state of polarization (see fig. 5). This region of maximum activity may be at relatively long wavelengths; rubidium 470$\mu\mu$, potassium 435$\mu\mu$, sodium 340$\mu\mu$, etc. Metals in general show photo-electric activity, their relative activity varying with surface conditions. Metallic compounds, especially sulphur and halogen compounds, show photo-electric action and are also extremely phosphorescent. Many non-metallic compounds, particularly many of the aniline dyes, are photo-electrically active. In metals, where absorption takes place in a surface film, electrons escape easily and a positive charge is left. This is also true of the photo-electric dyes in a solid form. In liquids, where the penetration is deeper, the electrons do not escape so easily and besides the emission of electrons, there is often an increase in the electrical conductivity of the solution. This happens also in some solids, of which selenium is the most noteworthy example. The true photo-electric effect (i.e., escape of electrons from the surface) is usually stimulated only by far ultra-violet light, which is absorbed in a surface film. Inner photo-electricity, or ionization due to light, which shows as an increased conductivity, is due to light of longer wavelength which penetrates into the liquid or solid in question, and the region of maximum activity may lie in the visible or infrared.

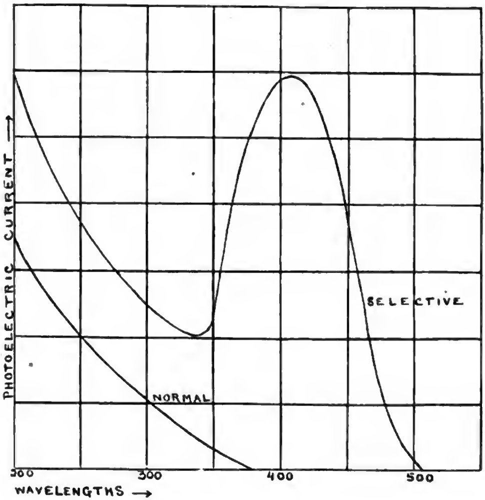

Fig. 5. Typical curves showing the normal and selective photo-electric effects.

The principal experimental results to be explained by theories of photo-electric action are these: *a*, uniform velocity of electrons for light of a given wavelength; *b*, velocity of electrons independent of intensity of light; *c*, number of electrons proportional to intensity of light; *d*, number and velocity of electrons increase with decreasing wavelength (i.e., increasing frequency) of exciting light; *e*, number and velocity of electrons independent of temperature; *f*, with increasing wavelength (decreasing frequency) the effect becomes 0 (i.e., there is a threshold frequency ν_0, such that no light of frequency less than ν_0 causes emission of electrons and any light of frequency greater than ν_0 causes some emission.) Many physicists prefer to believe that light acts through resonance, the energy of the orbital motion of the electron being gradually increased by sympathetic light vibrations until the electron is able to escape from the control of the forces binding it to the atom. Usually, however, the photo-electric effect is interpreted in terms of the quantum theory. It is supposed that the kinetic energy of an electron is increased by the energy of one light unit $= E =$ quantum of light energy $= h\nu$, where ν is the frequency of the light and h is a universal constant. If this whole energy is given to one electron, and none is lost by subsequent collision, it leaves the body with energy $= \frac{1}{2} mv^2 = h\nu - P$, where P is energy lost in getting out of the atom. The energy, hence the velocity, of the emitted electron thus obviously increases with increasing frequency (decreasing wavelength). Suppose $V = +$ potential just necessary to prevent the electron from leaving the body. Then $Ve = \frac{1}{2} mv^2 = h\nu - P$. The long wavelength limit of the photoelectric effect, or the photoelectric threshold, is that frequency (ν_0) for which the electron escapes with zero energy. It is therefore given by

$$O = h\nu_0 - P$$

so that $h\nu_0$ is the work required for an electron to escape from the atom and

$$Ve = \frac{1}{2} mv^2 = h(\nu - \nu_0)$$

This equation has been verified experimentally.

The existence of this threshold frequency (ν_0) is one of the most striking features of photo-electric action. It signalizes a sharp and absolute discontinuity in the phenomenon. It is a perfectly definite quantity when the condition of the body is definitely specified but it is extraordinarily sensitive to minute changes in surface conditions, such as is caused by the presence of extremely attenuated films of foreign matter. The importance of this change in threshold has only recently been appreciated, and so far few experiments have been done on it. A much larger number of investigations has been carried out on the change in the total photo-electric current (photo-electric sensitivity to unresolved light) under different conditions. It is very probable that these changes are due to a shift in photo-electric threshold, a shift toward the red making a greater portion of the spectrum active and thereby increasing the total current. Pohl and Pringsheim (74) found that the photo-electric threshold of newly distilled calcium amalgam moved from 350 to 600$\mu\mu$, and that of magnesium amalgam from 350 to 550$\mu\mu$ in 24 hours, without apparent cause. In the near future much more information on this important point will undoubtedly be available.

Throughout this paper, and throughout all the literature on light action, there has been continual reference to the phenomenon of fluorescence. This term is often vaguely understood. The term luminescence is a convenient one to include all cases in which there is an emission of light. We distinguish between electroluminescence, due to such agencies as cathode rays; triboluminescence, due to friction and crushing; chemiluminenscence, due to chemical action (to this type belongs the bioluminescence of fireflies and sea animals, which latter is unfortunately spoken of usually as phosphorescence); and photoluminesscence, due to light.

In electroluminescence we can see that the excitation must be closely connected with the displacement or separation of electrons from the atoms of the substance. In triboluminescence, bearing in mind the facts of frictional electricity, it is also probable that displacement or separation of electrons must take place. In chemiluminescence we have the rupture of a chemical bond (displacement of a valency electron). So when we come to photoluminescence it is natural to suppose that the separation, either partial or complete, of electrons from the atom is an important stage in the process by which emission of light is brought about.

Photoluminescence includes both fluorescence and phosphorescence. In both cases light of one wavelength, falling on a substance, results in the emission of light of another and longer wavelength. For instance, quinine shines with visible blue light when illuminated by ultraviolet. Luminescence observed only while the exciting light acts is called fluorescence, and that which continues after the stimulus ceases is phosphorescence. Generally speaking, liquids and vapors are fluorescent, and solids show both fluorescence and phosphorescence. Lenard and Saeland (75) have shown that phosphorescent solids are usually photo-electric and Stark and Steubing (76), in studying organic compounds, showed that those which fluoresce easily are generally photo-electrically active in a solid form. This is not easily observed in solutions since the emitted electrons are caught by the molecules of the solvent and do not escape, and the photo-electric effect becomes latent (77).

When exciting light falls on a fluorescent substance we may suppose that electrons are separated, partially or completely, from their parent atoms, but that this separation represents an unstable condition and recombination takes place with the emission of light. In the case of phosphorescence, a finite time elapses between the separation of the valency electrons and their return. Although fluorescence in general is accompanied by photo-electric action, this is not essential since the separation of the electrons may be only partial. We therefore can regard fluorescence and phosphorescence as evidences of a complete, or incomplete, separation of electrons under the action of light, with a subsequent return to the atom.

From the point of view of modern physics, we may regard it as practically certain that the first stage in any photochemical reaction consists in the separation of valency electrons under the influence of light. In the true photo-electric effect the electrons are lost from the substance, in fluorescence they return to their original atom, in photochemical reactions they attach themselves to some other atom, or group of atoms. In general, maximum fluorescence is given by wavelengths too long to produce any photo-electric action at all. In other words, under visible light the energy absorbed by an electron ($h\nu$) is too small to separate it completely from the atom. Pauli (78) studied the ratio of the intensity of fluorescent light to exciting light, and also the degree of photo-electric action, as a function of the wavelength of the exciting light. In the substances used, maximum fluorescence was stimulated by visible light from 400 to 500$\mu\mu$, whereas the photo-electric effect began in the ultraviolet and increased with decreasing wavelength. The two effects, however, overlapped over a considerable range. Thus, in the presence of very great photo-electricity, there would be little chance of fluorescence and photochemical action would be confined to a surface film. In cases of very strong fluorescence, with only a partial separation of electrons and a swift return to the original atom, there would be few electrons lost or joined to new atoms, and therefore little photo-electric or photochemical action. But at intermediate stages all three might occur simultaneously, and constitute three different possible manifestations of the displacement of valency electrons as the result of the absorption of light energy.

One photochemical reaction, which is universally familiar and of special use as an analogy in physiological actions, is the formation of an image on a photographic plate. Joly (79) is responsible for an explanation of this reaction which is a necessary step in the development of this theory. He advanced the theory that "the beginnings of photographic action involve an electronic discharge from the light sensitive silver halide molecule. In other words, the latent image is built up of ionized atoms or molecules upon which the chemical effects of the developer are subsequently directed." In support of this it is known that silver halides are vigorously photo-electric, with their activity in the descending order, bromide, chloride, iodide (the same order as their photographic sensitiveness). Also photographic images are produced by x-rays and radium, which are strong ionizing agents. It may be that the ionization brings about a chemical change and that the chemical product is the latent image, but as the latent image can be formed at temperatures approaching absolute zero, this explanation is not likely. If a gel containing silver halide grains is illuminated, the photo-electrons emitted move out with velocities depending on the wavelength of the light and, when illumination ceases, there is a charged grain surrounded by gel in which negative electrons are disseminated, the radius of the sphere of distribution depending on the velocity of the electrons emitted. The developer then reacts chemically with this latent image to effect reduction of the silver halide grains. On this theory a very complete explanation of photographic reversal is possible (80).

To this theory, as given by Joly, the following suggestion should be added which, so far as the author knows, has not been given before. An unsensitized plate responds only to short wavelengths, which is to be expected because, except for the selective effect, photo-electric action is given only by short wavelengths. After sensitization, plates respond to longer wavelengths. Since sensitizers are many of them known to be photo-electric it seemed at first that they might possess selective photo-electricity in the region of their absorption bands, thus making the plates photo-electrically active at longer wavelengths. But since the photo-electric activity of the sensitizers so far investigated is not of the selective type, this is not supported. It seems more likely that the sensitizers act by shifting the photo-electric threshold of the active silver halide particles to longer wavelengths so that they become photo-electrically active and capable of forming a latent image throughout the visible, and even into the infrared.

If we now consider the physiological actions of light on this theory we see that they fall in line very easily. For light of wavelength less than

$300\mu\mu$ we get intense reactions, due primarily we may suppose to the ionization of photo-active elements in the skin and blood of higher animals, and in the protoplasm of lower animals. This is analogous to the formation of the latent image in photography. Subsequent chemical action on these ionized substances produces changes which, in certain cells, result in the formation of the pigment melanin. In the particular case of pigment formation one might guess that tyrosin was the photo-active element involved. Besides pigment formation in the basal epithelial cells there are undoubtedly many other photochemical reactions induced by the ionized condition due to light. Ordinarily, light of longer wavelength is ineffective in producing physiological changes but the living cell, like the photographic plate, can be sensitized. In the presence of certain substances, such as eosin and hematoporphyrin, the photo-electric threshold of the cell, or of some of its constituents, shifts towards longer wavelengths and we get the same chemical changes initiated by visible light that we usually get only with ultraviolet. These changes naturally take place at the absorption bands of the sensitizer where the greatest amount of light energy is absorbed and made available for transformation into electronic energy. This shift in photo-electric threshold is easily brought about in vitro, giving rise to the many results obtained in photodynamic investigations. In vivo it happens only rarely, hematoporphyrin alone being capable of creating any marked sensitivity to visible light. There is considerable evidence to show that diseased tissue is more susceptible to radiations than normal tissue. To push the photographic analogy further, we may assume that normal tissue acts like a slow plate, and diseased tissue like a fast one.

In the green plant one may suppose that the chlorophyll produces a sensitized condition to visible light and makes it normally photo-active to long wavelengths, at the same time giving greater absorption in the visible. An analogy between chlorophyll and the sensitizers of a photographic plate has frequently been drawn (81). If, as has been postulated, the sensitizer acts by shifting the threshold toward the red, as well as by increasing the absorption in the visible, the plants without chlorophyll should show photo-synthesis in the ultraviolet. Experiments by Stoklasa (82) show that, in etiolated plants, the formation of chlorophyll proceeds at a greater rate under the quartz mercury arc than in sunlight. So it seems probable that the early stages of photosynthesis proceed faster in ultraviolet light and result in the formation of chlorophyll which then protects the plant against an overdose of ultraviolet, and sensitizes it so that synthesis then proceeds in the visible.

Reasoning along this line leads one to suppose that the pigment in human skin may act in a somewhat similar way, forming under ultraviolet light, and then acting as a sensitizer so that wavelengths greater than 300$\mu\mu$ become active in producing physiological effects. If this were true one would expect the good results of heliotherapy only after pigmentation had taken place. If, on the contrary, the chemical action resulting in the formation of pigment is the principal source of benefit, one would expect good results chiefly during the period of pigment formation.

The theory stated above can be summarized as follows. Light shorter than 300$\mu\mu$ acts on the living cell by ionizing its photo-electric constituents, and thereby leading to photochemical action. Light longer than 300$\mu\mu$ acts in the same way in the presence of sensitizers, which so affect the surface conditions of these constituents that their photo-electric threshold is shifted into the visible, and they therefore become ionized, with resulting chemical action, when illuminated by visible or near ultraviolet light.

This theory is based on purely theoretical considerations, but a recent paper by Schanz (83), written in support of a different view, contains experimental results which, although in no sense a proof, give more support to the theory here described than to that adopted by him. Schanz found that egg albumin is photo-electric and then examined its activity when combined with various photo-electric fluorescent dyes some of which are sensitizers and some not. Some of his results are given in table 5.

Schanz's explanation is that the sensitizers emit electrons which, by ionizing the molecules of the egg albumin, effect in it a chemical change. However, as none of the fluorescent substances used show any photo-electric effect for light through glass, there is no reason to suppose that they would by their own photo-electricity sensitize to visible light. If, however, the combination of egg albumin and a sensitizing dye resulted in shifting the photo-electric threshold of either toward the red, with resulting photo-electric action for wavelengths greater than 330$\mu\mu$, there would then be reason to expect photo-activity in the visible and near ultraviolet. The fact that Schanz found eosin and egg albumin together more photo-electric than either alone makes it probable that this actually occurred. Schanz however did not investigate the long wavelength limit of the effect and experiments are now in progress in this laboratory to test this point and to see whether, in general, substances sensitized so as to react in visible light, also show a shift in photo-electric threshold.

A number of scattered results give considerable evidence in favor of a photo-electric theory of photosynthesis. Becquerel (84) exposed collodion films containing silver bromide and chlorophyll to the solar spectrum and found reduction of the silver bromide in bands corresponding to the absorption bands of chlorophyll, and therefore to the region of photosynthesis. Dixon and Poole (85) found no photo-electric action in visible light in acetone extracts of pulverized leaves, as measured by the discharge of electrons from the surface. On the other hand Waller (86), Ries (87) and Samsonov (88) have all found that the Becquerel effect (production of a current by illumination of one electrode) was very marked when chlorophyll was used as the electrolyte. The maximum action in visible light was in the red, and there was no effect in the green, again corresponding to the region of photosynthesis. This tends, indirectly to support the general theory of light action given above.

TABLE 5

SUBSTANCE	LEAK OF ELECTROMETER IN 3 MINUTES
	Divisions
Fuchsin + H_2O	100.00
Fuchsin and egg albumin	40.00
Methylene blue + H_2O	100.00
Methylene blue and egg albumin	7.70
Fluorescein + H_2O	2.86
Fluorescein and egg albumin	2.63
Eosin + H_2O	1.18
Eosin and egg albumin	6.06
Egg albumin + H_2O	1.85

The importance of light in initiating chemical and physiological reactions has not been generally recognized in the past and perhaps should not be stressed too much now, without further experimental facts to substantiate it. But in closing, reference may be made to a remarkable paper recently published by Perrin (89) in which he boldly makes the hypothesis that all chemical reaction is initiated by the absorption of radiation (heat, light, etc.), and that the speed of the reaction is determined by the intensity of this radiation. He represents all chemical changes by equations of the type

$$A + h\nu = A' + h\nu'$$

Matter in a state of stable equilibrium A, on the absorption of a quantum of energy $h\nu$, goes into a state of equilibrium A' with the emission of a quantum of energy $h\nu'$, and vice versa for a reaction of a reversible type; $h(\nu - \nu')$ represents the heat of reaction, and for the particular reaction $3O_2 \rightleftarrows 2O_3$, which he considers in detail, where ν and ν' are the ultraviolet absorption frequencies of oxygen and ozone (wavelengths $165\mu\mu$ and $260\mu\mu$ respectively) the heat of reaction is actually closely represented by $h(\nu - \nu')$. On this theory all chemical change $A \rightarrow A'$ is initiated by the absorption of radiation of frequency ν and proceeds with the emission of fluorescent radiation of frequency ν', which may be in the infrared and hence invisible. This is only a hypothesis as yet but it leads to a satisfactory explanation of certain facts about the velocity of chemical reactions which were not explained on the older kinetic theory. Recent calculations by Langmuir (90) show that Perrin's theory does not apply to many cases of molecular dissociation in the simple form given above but suggest an additional source of energy for which the radiation may act as a releasing trigger. There is, however, in the paper a suggestion that photo-electric action in an enlarged sense may be one of the most fundamental and important occurrences in nature.

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TABLE 6

Emission spectra of various sources in the visible and ultraviolet

Sunlight	Continuous spectrum, except for the absorption of the Fraunhofer lines. Short wavelength limit = 291μμ, due to the absorption of ozone in the upper atmosphere.
Quartz mercury arc	Discontinuous spectrum giving a number of bright lines from 230μμ to 579 μμ. There are a number of weak lines below 230, the shortest being 185. The arc itself emits shorter lines which are absorbed by the quartz.
Electric sparks	Discontinuous. Many sparks give lines of shorter wavelength and greater intensity than the mercury arc. The aluminum spark gives strong lines at 185, 186, 193 and 199μμ.
Hydrogen discharge tube	Discontinuous. Relatively feeble in the visible but rich in very short wavelengths. If filtered through fluorite there are a large number of lines between 103 and 165μμ.
Carbon arc	Band spectrum, rich in near ultraviolet but weak beyond 300μμ, practically nothing below 240 μμ.
Iron arc	Very rich line spectrum with lines so close that it amounts almost to a continuous spectrum as far as 230μμ.
Tungsten arc	Richer in lines than the iron arc, especially good in the ultraviolet as far as 210μμ.

TABLE 7

Transparency limits in the ultraviolet

SUBSTANCE	LIMIT OF TRANSMISSION
	μμ
Fluorite (1-2 mm. thick)	125
Crystalline quartz (0.2 mm. thick)	145
Crystalline quartz (2.0 mm. thick)	150
Rock salt (2 mm.)	177
Clear quartz glass (fused silica)	180
Uviol glass	280
Common soda glass (0.2 mm.)	300
Common soda glass (2.0 mm.)	330
Water	190
Air	180

THE MECHANISM OF MUSCULAR CONTRACTION

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In 1914, the present writer prepared a review (33), for the Ergebnisse der Physiologie, on the relation between the heat-production of muscles and the chemical processes taking place in them. An account and bibliography, up to that date, of the work relevant to the present subject was given there, and it not proposed to cover this ground again, except insofar as more recent investigations require a revision of, or reference to, the earlier work. Neither is it proposed to discuss in general the theory of muscular contraction; the following pages are confined rather to an account of the various physical, chemical and mechanical phenomena exhibited by the active muscle, together with a speculation on their immediate causes. At present the phenomena themselves are complex enough, and ill-understood, and until we have an adequate foundation of fact it is worse than useless to erect an edifice of theory which will certainly topple down, as all theories of contraction have done before.

Part i. Mechanical phenomena. Evidence was given by A. V. Hill (28), (31), and by Hartree and A. V. Hill (23) (24), that it is simpler to regard the potential energy set free, rather the actual work done, as the mechanical end-product of physiological activity in muscle. This potential energy, which we will term the "theroretical maximum work," W_0, is widely different from W, the work actually realisable in practice. The difference is due to several factors: *a*, to the conditions of loading—if the load be too heavy the muscle cannot move it, and no work will be done: if it be too light the muscle cannot exert its full strength, and the work will be reduced: and between these limits every value of the load tends to waste some of the theoretical maximum work, W_0; *b*, to the fact that (in a twitch or a short tetanic contraction) relaxation may have commenced before the shortening is complete: and *c*, to the viscous resistance of the muscle to a rapid change of form. The actual work W is determined therefore by the manner and speed with which the shortening is carried out, while the theroretical maximum work W_0 depends only on the stimulus, and on the condition, temperature, and length of the muscle fibers. Hence, in comparing the mechanical with the thermal (or the chemical) response of muscle, it is desirable to employ the theoretical maximum work W_0 as the basis of comparison.

The theoretical maximum work. If the force exerted by a stretched elastic body be plotted against the distance through which it has been stretched, we obtain a "stress-strain diagram," the area of which gives the amount of work expended in the stretching. If the elastic body be one which does not show the phenomena of "after-extension," i.e., does not continue to strain when a constant stress is applied, the same amount of work can be recovered by allowing the body to shorten again. The theoretical maximum work W_0, or the potential energy, of the stretched elastic body, is then equal to the area of the stress-strain curve, and can be determined by experiment and calculation. If however the body, like rubber, be one showing the phenomena of after-extension, the work done in stretching will be equal to the work recoverable in shortening *only if both processes be carried out indefinitely slowly.* If either process be carried out at a finite speed, a finite proportion of the work, or of the potential energy, will be dissipated irreversibly by internal friction as heat. Muscle shows the phenomena of after-extension in an exaggerated form (see Hartree and A. V. Hill, (27)).

Suppose that a muscle be subjected to a stimulus of known strength and duration, and allowed to shorten freely to a length x, after which it comes up against a dynamometer of some kind, and the force f which it can exert isometrically at that length is measured. We thus obtain a curve relating force to length, and the area of this curve gives the theoretical maximum work W_0 of the excited muscle (31, p. 450; 56, p. 145). From this must be subtracted the work done in giving the muscle its initial extension, if any. The precise details of such a determination of W_0 are still a matter of dispute (see e.g., Meyerhof (56)), and no final conclusion can be said yet to have been reached: it is clear, however, that we are on the right road in seeking for an expression of some kind for the maximum work theoretically available in muscular activity. In any actual shortening, either of an active muscle or of a muscle passively stretched, the amount of work obtainable is less than this theoretical maximum, owing to the fact that the muscle can exert its maximum force at any length only if it be given a considerable time at that length to develop it. This is due to the same cause as the phenomena of after-extension in muscle, viz., to the viscous resistance of the muscle substance to a rapid change of form. (See fig. 1.) This phenomenon of after-extension is presumably due to the fact that muscle consists of a fine elastic network, containing a viscous fluid. Any change of shape necessitates a flow of fluid to a new position, and if a force be applied to the muscle it will be used, partly in stretching the network, and partly in driving the viscous fluid of the muscle through it.

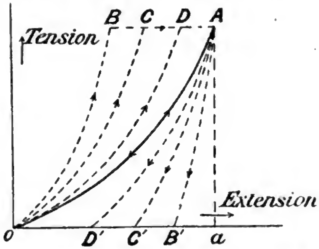

Fig. 1. Relation between tension and extension in a muscle. The full curve *OA* corresponds to a very slow process of unloading or loading the muscle from or to a given tension, and represents a "reversible" process. The broken curves represent "irreversible" processes carried out more or less rapidly. The curves *OBA*, *OCA* and *ODA* correspond to *loading* carried out rapidly, the most rapid being *OBA*, and the least rapid *ODA*. The curves *AB′O*, *AC′O*, *AD′O*, correspond to unloading carried out rapidly, the first being the most and the last being the least rapid. The potential energy possessed by the stretched muscle corresponds to the area *OAa*: the work done in stretching it rapidly along (say) the curve *OCA* corresponds to the area *OCAa*: the work obtained from it on unloading it rapidly corresponds (say) to the area *AC′a*: the work lost and degenerated into heat irreversibly in the complete cycle corresponds therefore to the area *OCAC′*.

(Note.—The curves are illustrative only and do not represent an actual observation.)

The realisable work. Most modes of contraction are inefficient. Either *a*, the load is too great and the muscle cannot finish its shortening; or *b*, the load is too small and the muscle cannot exert its full force; or *c*, relaxation has set in before the shortening is complete. In each case only a fraction of the available potential energy is realised as work: the rest is wasted as heat in the muscle. It is possible completely to avoid losses due to *a* and *b* by opposing the contractile force of the muscle, not to a load but to the inertial reaction of a mass: in this case the "reaction" of the mass adjusts itself exactly, neither more nor less, to the "action" exerted on it by the muscle, and the potential energy of the muscle is free to be transformed into the kinetic energy of the mass. By varying the size of the mass the time occupied in the shortening may be varied, and so adjusted that the shortening is complete before relaxation has commenced. In this way we may avoid all losses due to the factors *a*, *b*, and *c* above. There remains however one loss which we cannot eliminate, that due to the internal friction of the muscle substance itself.

Poiseuille's formula for the flow of a viscous fluid in a capillary tube states that the volume of fluid driven through per second is proportional to the pressure: in other words, the work done (and dissipated as heat) in driving a *given quantity* through is proportional to the speed with which the process is carried out. Turning to the case of a muscle, if the mass against whose reaction it pulls be large, and if its contraction be maintained, its speed of shortening will be small, and little of its potential energy will be wasted in overcoming its own viscous resistance to a change of form. Under such conditions the kinetic energy produced should approximate to the theoretical maximum work W_0. If, however, the shortening has to occur within a limited space of time, as e.g., in a twitch, the mass cannot be made very large, and the force exerted by the muscle at any length will be balanced, partly by the reaction of the mass, partly by the frictional resistance of the muscle substance itself to a rapid change of form. The latter factor will cause W, the actual work done, to be appreciably less than W_0, the elastic potential energy, the difference, $(W_0 - W)$, being dissipated as heat within the substance of the muscle. This has been shown in two ways: *a*, by Hartree and A. V. Hill (27, p. 165) on isolated muscles passively extended, the more rapid the shortening the less being the external work done: and *b*, by A. V. Hill (35) on human muscles, the proportion wasted, viz., $\frac{(W_0 - W)}{W_0}$ of the total potential energy developed, increasing with the speed of shortening according to the formula:

$$\frac{(W_0 - W)}{W_0} = \frac{k}{t}$$

where k is a constant depending on the viscosity of the muscle fluids, and t is the time occupied in the shortening. The work done can be expressed therefore in the form

$$W = W_0\left(1 - \frac{k}{t}\right)$$

This expression is very accurately true for the case of human arm-muscles.

The muscle twitch, especially at higher temperatures, lasts only a little time, and if shortening is to be complete before relaxation has commenced it has necessarily to occur rapidly. Consequently a considerable proportion of the theoretical maximum work has to be dissipated as heat. An investigation has been made by Doi (11), employing an inertia device (34) for measuring the maximum work, of the quantity $\frac{W}{Tl}$, W being the work realised in a maximal twitch, T the force developed in a maximal isometric twitch, and l the unextended length of the muscle. The value found was about 0.04 at 15°C., and was practically independent of the degree of extension. The calculation of the potential energy of the excited muscle, from the tension-length diagram (31, p. 453), gives a value for $\frac{W_0}{Tl}$ of about 0.14: hence in a twitch at 15° the realisable work W is only about $\frac{1}{3}$ to $\frac{1}{4}$ of W_0, the potential energy. Meyerhof (56, p. 154), employing analogous means, finds that the work in a twitch is always considerably less than that calculated from the diagram, especially in cases (e.g., rapid twitches at a high temperature) where theory requires a greater frictional degradation of energy. In prolonged contractions, on the other hand, he has shown that the work realised approximates to that calculated from the diagram. If the potential energy be not used up completely, either in doing external work, or in the irreversible viscous processes associated with the change of form of the muscle, in other words, if shortening be not complete when relaxation sets in, then what is left of the potential energy must somehow be dissipated as heat in relaxation. Hartree and A. V. Hill (23) have shown that there is a considerable evolution of heat during relaxation following an isometric contraction, and this would seem to represent—at any rate in part—the heat-equivalent of the potential energy developed during contraction.

If a muscle be given an unsuitable load it will waste its mechanical potential energy in one of two ways: if its load be too small it will move too fast and so dissipate too great a fraction of its potential energy in viscous processes inside it: if its load be too great it will move too slowly, or not at all, and will dissipate too great a fraction of its potential energy in relaxation. Thus there are really only *two* processes by which the theoretical maximum work W_0 can be wasted as heat, viz.: *a*, frictional loss, due to the rapid change of form; and *b*, relaxation. In any actual contraction both factors play their part.

Work collectors. Of such instruments Fick's "Arbeitsammler" (17, p. 140) is perhaps the best known. The same author described a "Winkelhebel" (17, p.55), and a "Schwunghebel" (17, p. 63) the principles of which have been used by Meyerhof (56). A. V. Hill (34), (27), (11) has described an inertia lever balanced on knife edges, for use with isolated muscles, and (35) a flywheel device for use with human muscles. All these instruments agree in principle in attempting to oppose the contraction of the muscle, at every stage, by a force just equal to the tension it can exert. In order to secure the greatest amount of work from a contracting muscle this principle is necessary, and should be embodied in any ergometer by the use of a suitable inertia, or otherwise. In the case of human muscular movements the conditions are apt to be somewhat altered by the fact that the mechanical "gearing-up" of the muscles, by their attachments in the body, may decrease as shortening proceeds, a fact which may make an isotonic contraction much more efficient than it is with an isolated muscle. The subject however of mechanical efficiency is dealt with below.

The isometric contraction. The actual shortening of a muscle is a complex process, involving a variety of physical and mechanical factors, in addition to the purely physiological ones concerned with the extent and course of the response. In order to eliminate these extraneous factors it is advisable, where possible, to deal with rigidly isometric contractions. Physiological literature is burdened with observations which depend rather on the properties of levers than on those of muscles, and it is possible to avoid any mechanical complication by employing an isometric spring-myograph, with good sensitivity and high natural frequency. (See (23 p. 115), (65, p. 245), (10).)

The effects of temperature. The rate of development of the isometric twitch has a high temperature coefficient, viz., about 2.5 for 10°C., and its rate of disappearance a still higher one, about 3.6 (25). These point to chemical reactions as the basis both of contraction and of relaxation. Theories of muscular activity have tended to neglect relaxation, or to minimise its importance, attributing it in some vague way merely to a reversal of contraction. Its high temperature coefficient, however, and other more direct evidence, force us (see p. 320 below) to regard it as a positive process, due to its own special chemical reactions, and in no way subsidiary to the other processes of contraction.

A rise of temperature affects not only the time-relations of a twitch but also its absolute size. At any but extreme initial extensions (10) the effect of a rise of temperature is to diminish the size of the twitch, in the same way as it diminishes its work (11), or its heat-production (12), (24). This probably arises from the fact that the amount of energy liberated in a twitch depends upon the *duration* of the change evoked by excitation, this duration being less at a higher temperature.

Initial extension. It was shown by Evans and A. V. Hill (16) that the force developed in an isometric twitch rises at first as the initial extension of the muscle is increased, reaches a maximum, and then rapidly decreases. The same is true of the heat-production. These experiments have been confirmed and extended by Doi (10), (11), (12), who has shown moreover that the maximum work exhibits the same dependence on the initial length of the muscle. Similar relations are true of the heart-beat, reading "pressure" for "force," "filling" for "extension," and "oxygen-intake" for "heat-production" ((10, p. 224); (37); (62); (47), (48); (60)). The speed also at which relaxation occurs is considerably decreased by an initial extension of the muscle (25), a fact which again is true of the heart (22). Finally, the temperature coefficients (25) of various factors in the twitch are affected by extension of the muscle. Of these phenomena, which are clearly of a very fundamental nature, no explanation can as yet be given: an extension of the muscle fiber from its natural resting length obviously modifies the surfaces or media in which the active processes of contraction and relaxation occur. The facts appear to be of great importance in relation to the heart.

Strength of stimulus. The time-course of the isometric twitch of a muscle directly stimulated is affected by strength of shock. The response to a weaker shock is invariably more prolonged than that to a stronger one, a fact difficult to explain on the "all or none" theory (25). (See p. 323 below.)

Recovery of contractility. The return of contractile power following a twitch has recently been studied by Adrian (1) and by Hartree and A. V. Hill (25). This "recovery" of contractility has nothing to do with the oxidative recovery process discussed below. If a muscle be subjected directly to two maximal shocks in succession, at a known interval, the effect of the second shock can be determined by comparing the response to the first shock only, with the combined response. Adrian found, in the case of cardiac muscle, that after a shock there is an absolute refractory period during which no response is given to the second shock, but that as the interval between the shocks is increased the response to the second shock increases to its original value, passing in the case of hearts perfused with an acid fluid, through a phase of supernormal contractile power, before settling down to its original state. In the case of skeletal muscle the time-relations are very different, and Hartree and A. V. Hill were able to demonstrate the same recovery of contractile power only by subtracting from the ordinates of the combined curve the ordinates of the curve produced by a single shock. If this be done it will be seen that, following a shock, the contractile power is at first nil, then rises to its original value again, passing invariably, however, in the case of skeletal muscle, through the supernormal phase. The ordinary so-called "beneficial effects of contraction" are entirely artificial, and due (1, p. 16) to the onset of fatigue and to recording instruments with too much inertia.

Tetanic contraction. The prolonged contraction is built up as the resultant of the responses to the individual shocks. The manner in which this building-up occurs has been discussed by Hartree and A. V. Hill (25), who find that successive elements of the response evoked by successive units of the stimulus differ from one another, becoming smaller and more prolonged as the stimulus proceeds, until finally a steady state is reached. The properties of the prolonged contraction are similar, in many ways, to those of the twitch. The effects of temperature on the time-course of the contraction are the same; on the size of the response, however, they are in striking contrast, a rise of temperature slightly decreasing the size of the twitch, but considerably increasing that of the prolonged contraction. The explanation of the latter effect is simple: the recovery of contractility following a twitch (see above) is much more rapid at a higher temperature, so that each element in the response is much less reduced by its predecessors. This factor more than counterbalances the slight diminution of the response to a single shock.

PART II. THE HEAT-PRODUCTION OF MUSCLES: *Methods.* There are two means of measuring directly the liberation of heat in muscles; *a*, the calorimetrical method for cases of prolonged heat-production; and *b*, the thermopile, for cases where the tissue is small and the heat-production of comparatively short duration.

The *calorimetrical* method involves the use of a good non-conducting container, such as a Dewar flask, and either a differential arrangement (29), or an accurately maintained thermostat (53, p. 258), in order to control loss of heat by conduction, etc. This method is suitable for the investigation of the heat liberated during prolonged stimulation, during survival with or without oxygen, during the onset of rigor, or during prolonged oxidative recovery. (See (29); (61); (53), (54); (58).) In order to quicken the oxidative recovery process Parnas used a cylindrical brass container, within which muscles could be kept in 2 or 3 atmospheres of oxygen, the container being placed in water in the calorimeter: a similar instrument was used by Meyerhof (54).

The *thermopile* is suitable for the investigation of the total heat produced in a contraction, of the time-relations of the heat-production, either "initial" or "recovery," and of the thermal changes associated with the passive lengthening or shortening of the muscle. In principle its use consists merely in placing a muscle in contact with the junctions of a thermopile, and recording the deflection of a sensitive galvanometer resulting from the rise of temperature. In practice a variety of precautions must be taken. In particular it is necessary to avoid differences of temperature at different parts of the instrument, or a gradual change of temperature of the whole instrument which will inevitably cause such differences: otherwise it is impossible to ensure a stable zero, or to make observations on muscles allowed to shorten (shortening may lead to warmer, or colder, points of the muscle coming on the junctions). Such thermopiles have been discussed by A. V. Hill (33) and by Bürker (6); recently Hartree and A. V. Hill (23), (26) have described modifications by which the zero may be maintained over a long period, the muscle subjected to any desired medium and allowed to shorten if required, photographic recording adopted, and quick and accurate calibration ensured.

As regards indirect methods of measuring the heat-production, apart from respiratory experiments applied to the whole animal, the only important method would appear to be the Warburg-Siebeck method, as employed by Meyerhof (54, p. 288), by means of which the oxygen absorbed by a single gastrocnemius of a frog may be accurately determined.

Thermo-elastic properties of muscle. According to Le Chatelier's rule, and to the Second Law of Thermodynamics, an elastic body suddenly subjected to a stress should show a thermal effect tending to neutralise the result of the stress. For example, if a steel wire be loaded suddenly its temperature will fall, because a fall of temperature causes the wire to shorten, and so tends to neutralise the lengthening produced by the load. These thermo-elastic effects are by no means small: they may easily be observed, and even employed in calculating the stresses in structures suddenly loaded (9). Realising that in the case of muscles allowed to shorten these thermo-elastic phenomena must necessarily be superimposed upon the other thermal effects accompanying contraction, Hartree and A V. Hill (27) investigated them experimentally in living muscle, in dead muscle, and in india-rubber. These substances, unlike most others, shorten on being warmed, so that extension should lead to a rise, and shortening to a fall of temperature. As seen in figure 2, extension of a muscle leads indeed to a rise of temperature, as predicted, while shortening leads at first to a fall, which fall however is rapidly absorbed in a subsequent rise. These phenomena were found to be due to two distinct processes, a reversible and an irreversible one. The reversible one is that predicted from thermodynamical theory and discussed above. The irreversible one is due to heat produced at the expense of mechanical energy, by viscous resistance to the change of form of the muscle (see p. 313). The viscous effect increases the thermoelastic effect in the case of extension, and decreases it in the case of shortening.

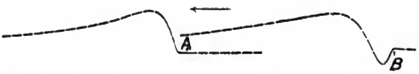

Fig. 2. Pair of sartorius muscles from *Rana temporaria.* Permanent load 5 grams At *A* on the left hand curve 155 grams were hung gently on the muscle: the temperature rose. At *B* on the right hand curve, after the 155 grams had been hanging on the muscle for some time, the load was gently removed: the temperature fell rapidly (the reversible effect) and then rose (the irreversible "viscous" effect) and finally fell again (the physical loss of heat by conduction). Time in seconds shown as gaps in the curves. Read from right to left.

These experiments, which were repeated under various conditions, confirm the conclusion that the external work done in a muscular contraction is diminished through viscosity by an amount depending upon the velocity of shortening (p. 313 above). Further, they are a necessary preliminary to certain investigations which have never yet been satisfactorily made, but for which the means are now available, and which are of the most fundamental interest; viz., those in which the muscle is allowed to shorten during the contraction, the heat-production being compared with the work directly measured, and the effect of the shortening on the total energy liberated being determined.

The "initial" heat-production. Under ordinary conditions, and provided that the time of stimulation be not too long, the maximum deflection of the galvanometer is an accurate measure of the total heat given out in the development, maintenance and disappearance of the mechanical response, i.e., in all phases except recovery. We shall refer to this heat as the "initial" heat. It was shown by Weizsäcker (67), (68) that the magnitude of the initial heat is uninfluenced by the presence or absence of oxygen, and by Hartree and A. V. Hill (23), employing a very sensitive method of comparison, that the time-relations of the initial heat are also quite uninfluenced by oxygen. Thus the "initial" breakdowns are entirely non-oxidative in character. It is clearly necessary to attribute two distinct mechanisms to the muscle, one similar to an electrical accumulator with an electromagnet and the necessary key and wires, by means of which chemical energy previously stored can be used either to do external work or to maintain a force as and when required, the other similar to a dynamo and a combustion engine, by which the accumulator may be recharged to its previous state after activity (23). The recovery process will be considered more fully in part III of this paper: here we shall deal only with the initial phases of contraction.

It has been shown by Hartree and A. V. Hill (23) that the initial process of contraction must be regarded as occurring in three phases, corresponding to the development, the maintenance and the disappearance (relaxation) of the mechanical response. By an accurate method of analysis of the photographic record of the galvanometer deflection, they proved that, in an isometric contraction, each phase is accompanied by a production of heat, as shown in figure 3. In the case of a single twitch, of course, the second phase (maintenance) is absent. It seems likely that the development of the response is due to the liberation of lactic acid, the lactic acid then proceeding to alter the tension and natural length of the muscle-fiber by some unknown effect of its hydrogen ion. In a twitch relaxation is then caused by the anaerobic chemical removal of the lactic acid from the site of its action. Both processes are accompanied by a production of heat. During prolonged stimulation the "maintenance" phase occurs, and must be accompanied by a continual evolution of heat, owing to the fact that a steady concentration of lactic acid can be maintained at its place of action *only* if the removal-processes be balanced by equal production-processes.

During the development of an isometric contraction heat, H_1, and potential energy, E, are produced in the muscle fibers: during relaxation the potential energy, E, disappears, either as heat, or into some latent molecular form, the lactic acid which excited its appearance being neutralised, or removed, with heat-production H_2, by some process unknown. The facts, a, that this process is accompanied by a production of heat, b, that the temperature coefficient of its velocity is very high, suggest that it is a positive chemical reaction, rather than a physical process such as diffusion away from the active areas (54, p. 310). If, moreover, the lactic acid, once it had done its work, simply collected by physical diffusion in the fluid spaces [Meyerhof's Ermüdungsorten] of the muscle fiber, then there should be a measurable increase of hydrogen ion concentration as the result of even a single twitch. Roaf (64) claimed to have demonstrated such a change by the use of an MnO_2 electrode, but Ritchie (63), repeating Roaf's experiments with great care, found that as the result even of a prolonged tetanus, the change of hydrogen ion concentration inside the muscle is almost negligibly small. Certainly the lactic acid produced in the muscle is neutralised very rapidly, and it is natural therefore to ascribe relaxation to this process of neutralisation.

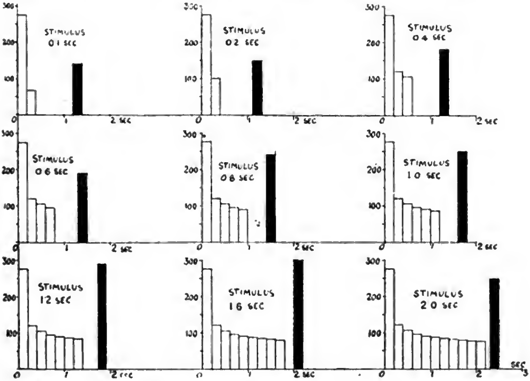

Fig. 3. Heat liberated by sartorius muscles at 0°C. in oxygen. The height of each rectangle represents the heat given out in the interval corresponding to the base on which it stands. Skeleton rectangles represent the heat liberated during contraction, black rectangles the heat liberated during or immediately after relaxation.

The isometric twitch. The total initial heat-production in a twitch, like the force developed or the maximum work done (see p. 316 above), is affected by extension of the muscle (16), (12), and by temperature (12), (24). In a maximal isometric twitch, as the initial extension increases the heat increases also, but only to a maximum, after which it diminishes considerably. It might be supposed that the energy-liberation is a surface effect, increasing with the length, or the surface, of the fiber; this, however, fails to explain the existence of the maximum, and the diminution with further extension. A rise of temperature diminishes H, the heat-production in a twitch, probably for the same reason as it diminishes T, the force developed (see p. 315 above).

The ratio $\frac{T}{H}$, in an isometric twitch, has been studied under a variety of conditions. The effect of temperature upon it seems to be very small; observations by Weizsäcker (67) appeared to show a definite effect, but Hartree and A. V. Hill (25) in carefully controlled experiments were unable to substantiate it. The effect, if any, is very small. Where nearly everything connected with muscular contraction is so largely influenced by temperature, the fact that $\frac{T}{H}$ is unaffected thereby suggests some special connection between T and H. Various observers have shown that there is a proportion between the oxygen-usage of a heart, and the pressure developed in its beats (62). Also, there are various grounds for believing that the potential energy developed is some fraction of Tl, l being the length of the muscle, (24), (31), (56). If so, the constancy of $\frac{T}{H}$ for varying temperature shows that the fraction of the total energy liberated as mechanical potential energy is independent of temperature.

The value of $\frac{T}{H}$ is affected by the strength of the shock, decreasing as the shock is increased (25, p. 402), and by initial extension, decreasing as the extension is increased (25, p. 400). Following a shock, the value of $\frac{T}{H}$ for a subsequent shock always passes through a prolonged supernormal phase (25, p. 406), the "efficiency" of a short tetanic contraction being thereby appreciably increased.

As the strength of the shock is increased the heat developed in a twitch, like the maximum force, increases in a series of steps until the maximal shock is reached; it is curious however to find (25, p. 404) that in some cases (but not in all) *a super-maximal shock evokes a smaller* *heat-production than a maximal one, although the mechanical responses are in size and character identically the same.* Some 15 per cent increase in efficiency therefore can be obtained sometimes by employing supermaximal shocks. The mechanism of this paradoxical result is quite unknown: its possibility, however, together with the facts, *a*, that the time-relations of the isometric twitch are affected by the strength of the shock; and *b*, that $\frac{T}{H}$ also is so affected, shows that there are distinct limitations to the "all or none" principle when applied to the twitch of a muscle fiber. If it were true under all circumstances that variation of strength of shock causes simply a variation in the *number* of fibers responding, and not in the character of their response, then none of these phenomena could occur (25, p. 404). Apparently even in a single twitch the response of a muscle fiber can be varied by varying the strength of shock directly applied to it, as well as by varying the extension and the temperature of the muscle; while the phenomena of summation of responses are well known. It seems unwise therefore to regard as a universal principle a rule, which may be generally true of nerve, but which in muscle is so restricted that it appears to have few points of application left.

The thermal response to a shock is not the *result* of a mechanical response, nor indeed is it necessarily accompanied by one. As Weizsäcker (67), (68) showed, the application of a suitable narcotic substance may eliminate the mechanical response completely, while leaving a considerable heat-production. Thus chemical reactions may be released by a shock even though there be no possibility of their evoking the visible changes of a mechanical response. This is good indirect evidence that the production of mechanical energy is due to some physical effect of one of the products, or intermediate products, of the reaction, the mechanism affected by that particular body being liable to be put out of action by the narcotic.

The prolonged contraction. The heat produced in a prolonged contraction, occurring in response to a maximal tetanic stimulus, depends upon a variety of factors, the initial extension, temperature and condition of the muscle, the frequency and duration of the stimulus, and the amount of shortening allowed. The last-named has never received adequate investigation, though the means are now available. All the following results refer to isometric contractions. The effects of initial extension are the same as in the simple twitch. The effects of temperature and duration are as shown in figure 4 (24, p. 137), for the unfatigued sartorius of *rana temporaria.* The heat-production increases with the frequency of stimulation, up to the point where complete summation is attained (32), after which it reaches a maximum and remains constant. Fatigue invariably causes a diminished heat-production.

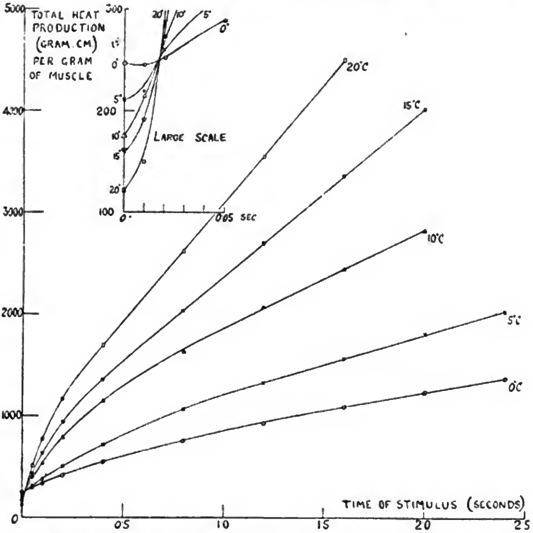

Fig. 4. Relation between heat-production, duration of stimulus and temperature. The large diagram shows the curves up to 2.0 seconds on a small scale; the small diagram shows the initial shape of the curves up to 0.05 second, on ten times the scale. Actual observations shown by dots.

Of these various factors the most important are shown in figure 4. For very short durations the heat is slightly greater at the lower temperature; for long durations it is much greater at the higher temperature; for one particular duration it is the same at all temperatures. (These experiments were made with a frequency of stimulation of 180 per second.) Moreover after a very short while the curves become straight lines; the heat-production occurs at a steady rate. The steady rate is unaffected by the frequency of stimulation, provided this has more than a certain value; its temperature coefficient however is 2.8 for 10°C., showing that some chemical reaction regulates the rate at which energy is supplied to maintain the contraction (24, p. 139). It seems possible that the immediate precursor of lactic acid, probably a hexose-phosphate, is present in its "ready" form only in very small amount, and has to be reformed from glycogen as required during prolonged stimulation. In that case the speed with which it is reformed would determine the rate of the energy discharge, and we should expect the latter to have a chemical temperature coefficient.

The energetics of prolonged stimulation may be expressed in another way, viz., by plotting $\frac{H}{T}$ (instead of H) against the duration of the stimulus (24, p. 144). If this be done it will be found that the total energy liberated in a prolonged isometric contraction can be split up into two components, one concerned in the *development* of the mechanical potential energy, the other in its *maintenance* at a constant level. Expressed in a mathematical form:

$$H = Tla\,(1 + bx)$$

where H is total energy, T is maximum force developed, l is length of muscle, x is duration of stimulus, and a and b are constants: a is independent of temperature, b is largely affected by it, increasing 2.3 times for 10°C. The quantity aTl represents the potential energy developed (or some quantity proportional to it); it is noticeable that the value of aTl agrees reasonably well with the potential energy calculated from the force-extension diagram of the active muscle. The quantity $bx(aTl)$ represents the energy degraded in a stimulus of duration x, in *maintaining* a state of potential energy aTl. The greater b is, the greater is the wastage of energy in maintaining a constant force. In a fresh, rapidly contracting, or warm muscle, b is larger; in a fatigued, slowly contracting, or cool muscle, b is smaller; thus in the latter cases the maintenance of a force is accomplished with the greater economy.

With certain limitations these results apply to human muscles. In the general expression for the total energy liberated:

$$H = aTl\,(1 + bx)$$

we may write $aTl = W_0$ (or some quantity proportional to W_0), where W_0 is the theoretical maximum work discussed above (p. 311). At a given temperature human muscle appears to contract much less rapidly than frog's, so that b is less; at 37° however b is large enough to make a prolonged contraction fairly uneconomical. It is shown below that in the complete cycle of the contraction, i.e., including the recovery process, there is about 2½ times as much energy set free as in the initial processes alone. Hence if Q be the total energy liberated in the complete cycle, we may write:

$$Q = 2.5\, W_0 (1 + bx) \ldots\ldots\ldots\ldots \quad \text{I}$$

This leads us to a consideration of the mechanical efficiency of human muscles. It is realised that equation I applies literally only to an isometric contraction: it will probably be found however to apply, with a different value of b, to a muscle undergoing actual shortening. The realisable work W, has been shown (p. 313) to obey the equation $W = W_0\left(1 - \frac{k}{t}\right)$, where k is a constant and t is the time occupied in the shortening. If the contraction be maintained only long enough to cover the actual shortening, i.e., if $x = t$, we may write $W = W_0\left(1 - \frac{k}{x}\right)$, so that the mechanical efficiency is

$$\frac{W}{Q} = \frac{W_0\left(1 - \frac{k}{x}\right)}{2.5\, W_0\,(1 + bx)}$$

$$= \frac{0.4\left(1 - \frac{k}{x}\right)}{(1 + bx)} \ldots\ldots\ldots\ldots \quad \text{II}$$

If we knew k and b we could calculate from equation II the mechanical efficiency of maximal human muscular movements carried out at any speed. A determination of k can be made as described by A. V. Hill (35). The value of b might be determined directly by measurements of oxygen-intake: it might also be inferred approximately from thermoelectric observations on small mammalian muscles; at present, however, it can only be arrived at backwards and roughly from a knowledge of the maximum net mechanical efficiency. Repeating the calculation given by A. V. Hill (35) with the rather different symbols and the new value of the recovery heat-production, it can be shown that the maximum mechanical efficiency is obtained for a value of $x = k\left[1 + \sqrt{1 + \frac{1}{klb}}\right]$ Putting $k = 0.24$, as found by Hill for the biceps and brachialis anticus of a healthy young man, the following table may be calculated:

b	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	1.0
Optimum x	00	1.82	1.37	1.18	1.05	0.97	0.92	0.88	0.84	0.79
Maximum W/Q	0.4	0.295	0.260	0.235	0.220	0.205	0.190	0.180	0.170	0.155

From this table, if we may assume the maximum net efficiency of human muscular movement to be about 0.26, the value of b is about 0.2. Taking this value for the sake of calculation (though the actual value makes no difference to the following argument) the efficiency of a maximal human muscular movement becomes

$$\frac{W}{Q} = \frac{0.4\left(1 - \frac{0.24}{x}\right)}{(1 + 0.2\,x)}$$

This relation between the efficiency and the time occupied in the movement, is shown in figure 5. It is seen that $\frac{W}{Q}$ rises rapidly from zero at time 0.24 second (the duration of an unloaded contraction), attains a maximum of 0.26 at 1.37 second, and then slowly falls again to zero. Thus human muscles have an optimum speed of working: a higher speed seriously interferes with their efficiency, a lower speed interferes indeed, but much less. It should not of course be supposed that the numbers given here are all absolute constants; they depend upon the characteristics of the actual muscles used; there can, however, be little doubt that the efficiency of human muscular movement is governed, in general, by considerations of the kind advanced above. It can be shown moreover (35) that a submaximal effort is less efficient than a similar maximal one, occurring in the same time against a heavier load. This is of importance as show the inadmissibility, in ergometer experiments, of adopting as "base-line" the energy expenditure at a lower level of work. (For example, see Benedict and Cathcart (3).) The whole subject would seem to be of considerable importance from the point of view of the optimum conditions for muscular efficiency in the processes of industry or athletics, and especially in relation to physiological experiments with ergometers. We cannot, however, discuss it further here.

An excellent short account of the *Thermodynamics of Muscles* has been given by Meyerhof (51).

PART III. THE RECOVERY PROCESS. *Isolated muscles.* The discoveries a, of Liebig (42) that an atmosphere of oxygen preserves the irritability of an excised resting muscle, and b, of Ludwig and Schmidt (45) that oxygen delays the onset of fatigue in a similar excited muscle, were followed by the proof by Fletcher (18) that a muscle may be preserved indefinitely from the onset of rigor mortis, or indeed recalled from its incipient stages, by immersion in oxygen. These researches, culminating in the classical investigation by Fletcher and Hopkins (20) of lactic acid in muscle, led the present writer (30) in 1913 to determine the heat-production during the recovery phase. It was found, in the presence of oxygen, that the production of heat is prolonged for some minutes after excitation, the total "delayed" heat being of the same order of magnitude as the "initial" heat; while, in the absence of oxygen, the delayed heat-production was much smaller, or (as was supposed then) negligible. This, together with the proof that the "initial heat" has a non-oxidative origin, makes it clear that the muscle is similar to an accumulator, that it contains potential energy ready for discharge, which is restored by some oxidative process during recovery. What is the nature of this recovery process, and is it the oxidation of lactic acid?

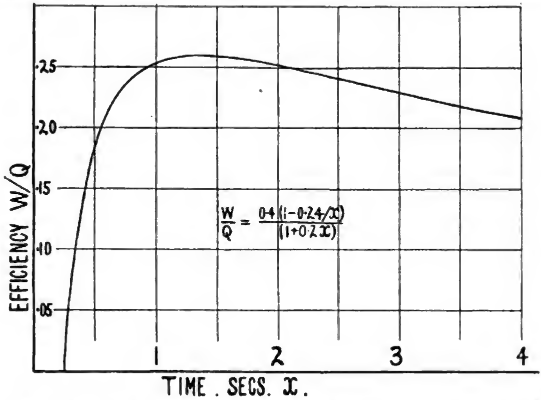

Fig. 5. Mechanical efficiency of human muscular movement in relation to the duration, x, of the contraction.

Experiments by A. V. Hill (29) and by Peters (61) had proved that the production of 1 gram of lactic acid in muscle, whether in rigor or as the result of exercise in the absence of oxygen, is accompanied by the liberation of about 450 calories. Later and more exact experiments by Meyerhof (53) have shown that this value is rather too high, that in rigor, or in fatigue induced by a succession of single shocks or short tetani, the value should be about 350 calories[1] per gram of lactic acid; the difference does not affect the argument. If, as A. V. Hill (30) estimated, the oxidative recovery process involved about as much heat-production as the initial process, the total heat set free in the complete cycle of liberating and removing 1 gram of lactic acid had to be about 2 × 450 = 900 calories; later and much more accurate observations by Hartree and A. V. Hill (26) have shown that the average value of the total recovery heat is about 1.5 times the initial heat; accepting this figure, together with Meyerhof's more accurate determination, the total energy liberated in the complete cycle is 2.5 × 350 = 875 calories. This is close to the 900 calories originally estimated. Thus in the liberation and the subsequent oxidative removal of 1 gram of lactic acid 875 calories have been produced, and the muscle is finally in the same state as before, except that some of its glycogen has been oxidised. The heat of combustion of 1 gram of lactic acid is 3661 calories, while that of the corresponding amount (0.9 gram) of glycogen is 3772 calories;[1] the total heat liberated in the production and the subsequent oxidative removal of 1 gram of lactic acid is only 24 per cent of the former and 23 per cent of the latter. It was obvious therefore that, unless there were some serious error, the whole of the lactic acid was not removed by the simple process of oxidation, that for every 1 gram of lactic acid oxidised in the recovery phase, some 3 more grams of lactic acid were removed by some other non-oxidative mechanism. There seemed no escape from this conclusion. In 1915, however, Parnas (58) published experiments purporting to show that during the oxidative recovery phase a complete equivalence existed between the oxygen used and the lactic acid removed. These experiments were accepted by Fletcher and Hopkins (21) in their Croonian Lecture, and at first by Meyerhof (52), and it was assumed in consequence that in the recovery phase the whole of the lactic acid was really oxidised, and not, as had been supposed, restored to its previous position ready for subsequent acitvity. There remained, however, the fundamental difficulty that on this view the recovery heat-production was quite inadequate. From this impasse the situation has been relieved by the brilliant investigations of Meyerhof. Meyerhof first (54) repeated the experiments of Parnas with entirely the contrary result. Allowing for the oxygen used in the same period by similar resting muscles he found that *during the recovery period of stimulated muscles an excess of oxygen is used which is insufficient to account for the oxidation of more than ⅓ to ¼ of the lactic acid which can be shown, by direct determination, to have disappeared.* This is in excellent agreement with the results of the calculation from the recovery heat-production. Meyerhof repeated his measurements of the oxygen used in the recovery process under various conditions, always with the same result, viz., that not more than ⅓ to ¼ of the lactic acid is removed by direct oxidation. It is difficult not to attribute Parnas' results to some unknown experimental error. Meyerhof moreover has confirmed his results by a variety of other observations. In the first place he found (52) that in the recovery removal of lactic acid the volume of CO_2 produced is almost exactly equal to that of O_2 absorbed; the respiratory quotient is unity. This points to the oxidation either of lactic acid or of carbohydrate. Further, by direct measurement (in a calorimeter) of the recovery heat-production after prolonged stimulation, he found (54) that the heat is less than that calculated from the oxygen used by an amount equivalent to the energy liberated in the initial stages of the contraction; in other words, the energy liberated by oxidation in the recovery process partly appears as heat, and partly is absorbed in some physico-chemical process restoring the muscle to its original condition. Thus the heat set free plus the energy re-absorbed is shown, by direct measurement, to correspond to the oxygen used; and this oxygen corresponds to the oxidation of an amount of lactic acid only ⅓ to ¼ of that known actually to have been removed. Finally Meyerhof has shown (54), (55), again in contradiction of Parnas (58), that (making due allowance for the oxidation of a quantity corresponding to the measured amount of oxygen used in survival or recovery) whenever lactic acid appears a corresponding amount of glycogen disappears, and that whenever lactic acid disappears a corresponding amount of glycogen can be recovered. It seems therefore quite certain, *a*, that the recovery process consists of an oxidation (either of lactic acid or of carbohydrate), of which part of the energy appears as heat, part is absorbed in restoring the muscle to its original condition of readiness for mechanical activity; *b*, that in the initial process of contraction glycogen, or some product of glycogen, is changed explosively into lactic acid; *c*, that in the recovery process the glycogen (or its product) is restored and the lactic acid removed; and *d*, that the oxygen used in recovery is employed in oxidising carbohydrate (or lactic acid) in amount equivalent to about ¼ of the lactic acid removed.

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[1] Since this article was completed the writer has received the manuscript of a forthcoming article by Meyerhof, in which this figure is modified slightly. See Appendix.

Experiments have recently been made by Hartree and A. V. Hill (26), with particular care to ensure accuracy, to determine both the extent and the time-relations of the recovery heat-production. The rate of recovery heat-production appears to follow a perfectly definite time-course, as shown in figure 6; it starts at a low level immediately following the contraction, rises to a maximum, and then slowly falls to zero, the last part of its curve being of an exponential character. It is noticeable that the longer stimulus, evoking the more extensive liberation of energy, gives a recovery heat-production which is not only absolutely but relatively more rapid than that given by the shorter stimulus. When the velocity of a chemical reaction increases more rapidly than the first power of the concentration of one of its reacting

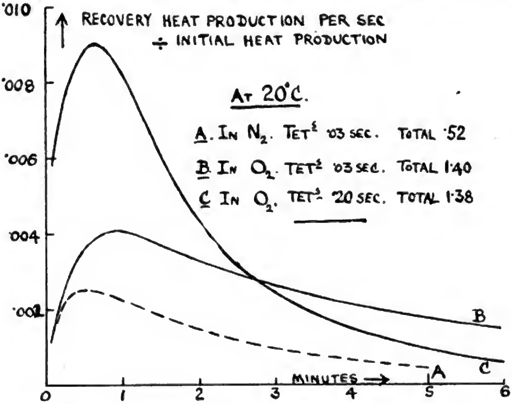

Fig. 6. Rate of production of heat during recovery of sartorius of *rana temp.* in O_2 and in N_2. Note that the heat is expressed, not in absolute units, but as a fraction of the initial heat-production.

bodies we conclude that the "order" of the reaction is greater than unity, that we are dealing with a bimolecular or a termolecular reaction. All the experiments made confirm the conclusion that the "order" of the reaction occurring in the recovery process is greater than unity, a conclusion which is entirely in keeping with the view advanced by Meyerhof (54), on the basis of Embden's work, that in the recovery process two molecules of lactic acid are united to make one molecule of glucose, which is then combined with phosphoric acid to form a hexose diphosphate. The rate of the recovery heat-production is also affected by temperature, increasing rapidly as the temperature rises. The cause of the maximum on the curve, until we have further knowledge of the chemical reactions occurring in recovery, must remain a matter of conjecture; were these chemical reactions of a simple kind we should expect an initial high rate followed by a continuous fall to zero: clearly we are dealing here with complex reactions whose full activity is not manifest until some time after their commencement. Possibly the time-relations of the heat-production during recovery may give some clue as to the actual chemical mechanism of the latter.

In the experiments of Hartree and A. V. Hill (26) the special precautions taken to ensure a steady zero made it possible to measure the recovery heat-production after a short tetanus (0.3 to 0.5 second), for some 6 or more minutes, and so to arrive at an accurate estimate of its total value. Calling the total initial heat-production H_0 and the total recovery heat-production H_r, the ratio $\frac{H_r}{H_o}$, in frog's muscles in oxygen at 20°C., had values varying from 1.0 to 2.0, (the majority lying between 1.4 and 1.6) and a mean of 1.5. The ratio was independent of the duration of the stimulus. In the absence of oxygen, i.e., after some hours in O_2-free nitrogen, with preliminary stimuli to use up any traces of oxygen still present, the delayed heat-production was not abolished altogether but was still perceptible as shown in figure 6. The total value of the delayed heat in this case bore to the initial heat a ratio varying from 0.3 to 0.6, with a mean of about 0.4. This might perhaps be attributed to oxygen still present dissolved in the muscle, although all precautions had been taken to avoid this possibility; but a thin sartorius muscle soaked in a KCN solution (0.0007 n to 0.002 n) for 1 minute or more, in which presumably all oxidations had been eliminated, still showed a delayed heat-production amounting to some 30 per cent of the initial heat. If further investigations confirm the existence of this smaller anaerobic delayed heat-production we shall have to assume either

1, that the chemical reactions of the recovery process can progress to some degree in the absence of oxygen, and get held up only at the oxidative stage; or *2*, that in the muscle there exists some hydrogen acceptor capable, like the dipeptide isolated by Hopkins (36), of allowing oxidations to proceed in part even in the absence of molecular oxygen; or *3*, that the heat liberated in the absence of oxygen should be regarded as belonging to the initial stage (possibly relaxation), and not to the recovery stage at all. There seems at present to be insufficient evidence to allow us to distinguish between these three alternatives.[2]

The existence of this anaerobic delayed heat-production may upset some of the calculations given above. In anaerobic activity, or in rigor, the heat measured by Meyerhof, for comparison with the lactic acid formed, is the same as the sum of the initial and the delayed heats measured by Hartree and Hill in the absence of oxygen. Taking the "initial heat" as 100, the delayed heat in the absence of oxygen is 40, while the recovery heat in the presence of oxygen is an extra 110. In this case the total energy[2] of the oxidative phase is not 2.5 times, but only 1.8 times the total heat of the combined anaerobic phases. This will only strengthen the argument in favor of the non-oxidative removal of lactic acid, but without further evidence as to the mechanism of this post-activity non-oxidative heat-production it will be useless further to discuss the matter here.[3]

Unisolated muscle. Verzàr (66), observing the oxygen-usage of the gastrocnemius of an anesthetised cat, found that during stimulation the rate of oxygen-intake is not increased, but that after stimulation it rises and remains for a long time above its resting level. The reason for the absence of a rise during stimulation is presumably, as Lindhard (43) found for man during "static work," that the blood supply is hindered by the hardening of the active muscle. Barcroft and Kato (2) performed similar experiments on the gastrocnemius of anesthetised dogs, employing rhythmic instead of continuous stimulation. They found a rise of oxygen-intake during stimulation (the blood supply not being stopped by rhythmic excitation) but a still greater rise lasting for hours after the stimulus was over. These experiments contrast curiously with those on man (see below), in that the recovery process is so prolonged; after several hours the return to resting level is not as complete as it is, in man, after as many minutes. Whether this be due to excessive stimulation, or to the effect of the anesthetic, one cannot say. There is no doubt, however, that such experiments do not represent the normal processes of recovery in warm-blooded animals, which are better studied in man.

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[2] That is, heat plus restored potential energy.

[3] See Appendix.

Man. Owing to the lag in a calorimetrical method, and to the errors inherent in calculation from the CO_2-output during a rapidly changing metabolsim, the only practicable means of following the recovery process in man is that of determining the oxygen-intake. In their experiments on Pike's Peak (13, p. 258) Douglas, Haldane, Henderson and Schneider made preliminary observations, by the Douglas bag method, of the course and extent of the oxygen-intake during recovery from exercise in man. These experiments have been confirmed and extended by Campbell, Douglas and Hobson (7), and recently by Lupton (46), also using the Douglas bag method, the former investigating the recovery period after work on a bicycle ergometer, the latter after various other forms of exercise. Krogh and Lindhard also (40) have investigated the oxygen-intake after ergometer exercise, and Lindhard (43), (44) after the "static work" of supporting the body in various positions, customary in a gymnasium. In healthy men recovery is very rapid, the primary return to a resting level of oxygen-intake being complete within 10 minutes of the end of exercise, and almost complete within 3 or 4 minutes. Even after an exhausting effort recovery is not sensibly prolonged, though there may be a small increase in the resting level of oxygen-intake, corresponding possibly to a rise of body temperature, possibly to a restoration of glycogen in the muscle.

At 20°C. the recovery heat-production of a frog's sartorius in oxygen is nearly complete in 6 minutes (see fig. 6), 50 per cent complete within 2 minutes. Hartree and A. V. Hill (26) found the recovery heat-production to be considerably affected by temperature, though they could not determine a temperature coefficient. Assuming the latter to be about 2.4, the recovery process at 37°C. should be nearly complete within 1½ minutes, and 50 per cent complete within 30 seconds. In man the oxygen pressure is not so high, and we might expect the rate of recovery to be less. Hence the speed of oxidative recovery is of the same order of size in man as in isolated muscle. After prolonged stimulation of large masses of isolated muscle the speed of recovery is determined by the rate of oxygen diffusion, and is therefore very slow; where, however, the oxygen supply is adequate, and especially at a high temperature, oxidative recovery is a fairly rapid process.

Muscular exercise may be long continued, or short, while the chemical processes of recovery may occur mainly during the exercise, or wholly after it. At the beginning of exertion an oxygen deficit is built up which is compensated only at the end. In prolonged exertion the greater part of the recovery process is completed during the exercise itself; during uniform exertion a steady state is rapidly reached in which the total oxygen deficit becomes constant. In this case the total oxygen absorbed in the post-exercise recovery process is equal to that used in not more than ½ to 1 minute of the previous exertion. This shows the extreme rapidity of the oxidative recovery process in normal man. The highest possible rate of oxygen-intake in man, during steady exercise, is about 6000 cc. per minute; a total therefore of something like 4000 cc. may be used in recovery. Assuming that 22.2 liters of oxygen oxidise 30 grams of lactic acid or glucose, and correspond therefore (from above) to the removal, during recovery, of about 120 grams of lactic acid, we see that during and immediately after severe exercise, there must be something of the order of 22 grams of lactic acid present in the body. If the active muscles be assumed, for the sake of calculation, to weigh 16 kilos, this corresponds to a lactic acid concentration of about 0.13 per cent, an amount which is about ½ of that found in isolated frog's muscles after severe anaerobic stimulation. These 22 grams of lactic acid we may suppose neutralised by bicarbonate present in the blood or muscles; in the process it must turn out some 16 liters of CO_2, while during its oxidative removal in recovery the same amount of CO_2 must finally be retained by the body. It is small wonder, therefore, either that lactic acid may appear in the blood and urine, or that the combined CO_2 of the blood may be altered (8), or that the respiratory quotient may exhibit violent fluctuations, as the results of severe exercise and during recovery therefrom (7, p. 36); (46). These fluctuations must render any attempt to follow the rapidly changing metabolism in such cases, by means of measurements of the CO_2 output, entirely useless.

Lupton (46) has investigated also the recovery from short bouts of very violent exercise, of a nature too severe to be prolonged. In the extreme case of 10 seconds of such exercise, during which the subject holds his breath, practically the whole of the oxygen used after exercise in excess of the resting level, must be due to recovery, and Lupton found values as high as 2000 cc., as the total oxygen usage of recovery from 10 seconds exercise. This corresponds to a production of about 11 grams of lactic acid, a concentration roughly of the order of 0.05 per cent averaged over all the muscles of the body. This amount of oxygen used in the oxidation of carbohydrate yields about 10,000 calories, enough to raise the body-temperature of an 80 kilo man some 0.15°C. Thus the most extreme form of exercise causes, per second of the exercise, a total liberation of energy of 1000 calories. This, if continued, would be equivalent to an expenditure of 4.18 kilowatts or of 5½ horse-power, a rate which it would clearly be impossible for a man to maintain for more than 20 or 30 seconds. The total oxygen dissolved in the fluids of the body of an 80 kilo man, at the resting venous oxygen pressure, say at 50 mm. Hg, is about 100 cc.; the total oxygen combined with hemoglobin in his blood is not more than 800 cc.; while the rate of oxygen supply by the circulation, even after it has been pushed to its extreme limit by continued exertion, cannot much exceed 6000 cc. per minute. Consequently the most violent exercise of which a man is capable may in 5 seconds lead to breakdowns requiring recovery oxidations sufficient to exhaust all the molecular oxygen available in the body; and since such exertion may be continued certainly for 20 seconds the body may, so to speak, go into debt for oxygen to the extent of 3000 or 4000 cc. It is this fact alone which makes it possible for the body to carry out the more violent forms of exertion; were it necessary for the oxygen supply to keep pace with the exertion the capacity of the body for short-lived violent effort would be reduced to one-half or less. The same fundamental importance of recovery oxidations is shown by the experiments of Lindhard (43), (44). Here the subject, for example, maintained his body-weight for about 1 minute with arms bent, the rate of oxygen-intake being measured during, and at various moments after the exercise. In one experiment the total increased oxygen-intake, as the result of the exercise, was 1125 cc., of which only 168 cc. were used during the working period itself. Lindhard explains the lowness of the oxygen-intake during the working period as due mainly to the rigidity of the active muscles placing a severe restraint upon the circulation in them, and so restricting their oxygen supply. The fact that nearly 1000 cc. of oxygen are required in the recovery of what is really a very limited mass of muscles, sufficiently explains the extreme fatigue occasioned by this type of "static work;" this quantity of oxygen is required to remove about 5½ grams of lactic acid; the muscles involved probably do not weigh more than 2 kilos; consequently a concentration is attained of some 0.2 per cent to 0.3 per cent—somewhere near the maximum amount attainable by stimulation.

PART IV. THE PLACE OF LACTIC ACID IN THE MECHANISM: The *lactic acid maximum* established by Fletcher and Hopkins (20), the fact that the heat-production associated with the anaerobic formation of lactic acid shows the same phenomena (29), and that this heat-production is

3 to 4 times[1] as great as that obtainable from the breakdown of glycogen into lactic acid, led various writers (and especially the present one) to assume the existence in muscle, in limited amount, of some lactic acid "precursor" possessing stores of energy available for release in muscular activity, this precursor being restored during oxidative recovery (33). Recently however evidence has accumulated that the lactic acid maximum is due to a process of "self-inhibition," the formation of acid being stopped by the rising hydrogen ion concentration (41), (53). If the rise of hydrogen ion concentration be prevented in any way, e.g., by alkali, or by diffusion of the lactic acid into surrounding Ringer's solution, the formation of acid may reach a considerably higher level. The same thing is borne out by the experiments of Meyerhof (56), who found the total mechanical response (i.e., to complete fatigue), as also the acid maximum, to be increased in an alkaline medium. Hence one aspect of the case for such a type of lactic acid precursor completely disappears. Moreover the evidence has become overwhelmingly strong[4] that lactic acid arises, possibly not directly, from glycogen (59), (55), and the work of the Embden School (14), (15), which there is no space to discuss here, seems to show that some hexose-diphosphate is a link in the chain by which glycogen and lactic acid are mutually transformable into one another. According to Meyerhof (55), the reaction may be formulated as follows (writing glycogen as $(C_6H_{10}O_5)_n$).

Anaerobic breakdown

$$
\begin{aligned}
&5/n\ (C_6\,H_{10}\,O_5)_n + 5\,H_2O + 8\,H_3\,PO_4 \\
&\longrightarrow 4\,C_6\,H_{10}\,O_4\,(H_2PO_4)_2 + C_6\,H_{12}\,O_6 + 8\,H_2O \\
&\longrightarrow 8\,C_3\,H_6\,O_3 + 8\,H_3\,PO_4 + C_6\,H_{12}\,O_6
\end{aligned}
$$

Oxidative recovery

$$
\begin{aligned}
&8\,C_3\,H_6\,O_3 + 8\,H_3\,PO_4 + C_6\,H_{12}\,O_6 + 6\,O_2 \\
&\longrightarrow 4\,C_6\,H_{10}\,O_4\,(H_2PO_4)_2 + 6\,CO_2 + 14\,H_2O \\
&\longrightarrow 4/n\ (C_6\,H_{10}\,O_5)_n + 8\,H_3\,PO_4 + 6\,CO_2 + 10\,H_2O
\end{aligned}
$$

If this be so we are left with one fundamental problem, the fact that the heat-production in the anaerobic phase is much greater, and in the oxidative recovery phase much less, than corresponds to the chemical reactions written down. Landolt and Börnstein give the heat of combustion of glycogen as 4191 calories per gram, and of lactic acid as 3661 calories.[1] Thus 0.9 gram of glycogen forms 1 gram of lactic acid with the evolution of only 109 calories, an amount only ⅓ to ¼ of that actually appearing in the anaerobic formation of 1 gram of lactic acid in muscle; there is an excess of some 250 calories to account for. In the recovery phase there is a similar deficit. It is natural to attribute this alternating excess and deficit to the forward and backward progress of some reaction directly connected with the mechanical response. In the complete anaerobic cycle of an isometric contraction and relaxation no external work has been done and nothing has happened, so far as we know, except that a certain amount of lactic acid has appeared. Any action of the lactic acid, or its hydrogen ion, on sensitive surfaces (Verkürzungsorten (Meyerhof)) during contraction has been reversed during relaxation, a conclusion which is borne out by the fact that relaxation is accompanied by heat-production (23). Everything is as it was, except that some glycogen has disappeared and some lactic acid has appeared, not indeed free acid, as Ritchie (63) has shown, but acid in some combined or neutralised form. There seems no escape from the conclusion that the combination, or neutralisation, of the acid is responsible for the extra 250 calories appearing in the anaerobic phase, and that the reversal of this combination, or neutralisation, is responsible for the similar deficit in the oxidative recovery phase (53, p. 273).

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[4] Since the above was written a paper has appeared by Foster and Moyle, Biochem. Journ., 1921, xv, 672, which strongly confirms this conclusion. See also Hopkins' Herter Lecture, No. 2, Johns Hopkins Hosp. Bull., 1921, xxxii, 359.

The neutralisation of lactic acid with bicarbonate involves only a very small quantity of heat, according to an estimation by A. V. Hill (33, p. 371) only about 27 calories per gram of lactic acid. This is not sufficient to account for the excess and deficit under consideration. The neutralisation of the acid with free base (KOH) would supply us with enough heat, but there is no free base present. It may be, however, that in the fluids of the muscle there is some alkaline protein body capable of combining with and neutralising the lactic acid with the required evolution of heat.[2] In any case the process by which the lactic acid is fixed must be one possessing considerable free energy, since it has to reverse the very vigorous one in which lactic acid reacts with the sensitive surfaces of the muscle, producing free energy in the mechanical form.

In this way the vain search for a lactic acid "precursor," possessing stores of potential energy ready for subsequent release, has been replaced by an attempt to find the chemical mechanism of relaxation, the means by which lactic acid, having effected the development of tension, is removed from the site of its action, so allowing the previous state to reappear.

In a recent summary (38) J. v. Kries has sketched the following picture of the manner in which muscular contraction occurs: *a*, *contraction* is due to physico-chemical forces employing a form of energy present in amount only sufficient for one maximal contraction; *b*, in *relaxation* this form of energy is restored by forces which may be described as the affinity of some intermediate body for lactic acid; this body is present in amount sufficient to produce relaxation for a number of contractions; and *c*, in *recovery*, the energy of the intermediate body is restored by oxidative processes. It will be seen that this sketch follows very closely the lines suggested above.

An excellent short account of the rôle of lactic acid in muscular contraction has been given by Meyerhof (50).

APPENDIX

Since the above pages were completed the writer has been able to see the manuscript of a paper by Meyerhof (Energieumwandlungen VI) shortly to appear in Pflüger's Archives. The conclusions in this paper are so pertinent to the discussion here that they have been included in this appendix. First, Meyerhof has redetermined the heat of combustion of lactic acid, and finds it appreciably different from that given in books of chemical tables. He finds that the formation and neutralization (with phosphate or bicarbonate) of 1 gram of lactic acid from glycogen leads to an evolution of about 190 calories, a considerable increase on that estimated above. He finds also that in intact muscle the same amount of lactic acid is formed anaerobically from glycogen with an evolution of about 370 calories. If the acid be allowed to escape into a surrounding alkaline fluid, this value is considerably reduced, showing that something more than neutralization with an alkaline salt occurs in the living muscle. If lactic acid be formed from glycogen in crushed muscle the heat corresponds closely to the 190 calories, showing that here we have merely the formation and simple neutralization of lactic acid. If moreover an acid be allowed to penetrate into a muscle, without the liberation of lactic acid, the heat produced is about half of that accompanying an equal production of lactic acid in the live muscle. It is clear therefore that the presence of the lactic acid in the muscle leads to an evolution of about 180 calories beyond that caused in its production from glycogen. Meyerhof supposes that this arises from the heat of dissociation of the muscle proteins, and he has shown that, although the neutralization of 1 gram of lactic acid by phosphate or bicarbonate yields only about 20 calories, the addition of the same acid to buffered amino acid solutions yields some 6 or 7 times as much heat.

It is very probable therefore that the remaining $370 - 190 = 180$ calories must be attributed to some reaction between the acid and the protein constituents of the sarcoplasm. If this reaction be a slow one it may account for the delayed anaerobic heat shown in figure 6: and in this case the process of relaxation may be ascribed to the immediate neutralization of the acid by alkaline salts, the acid then being slowly removed by the proteins, thereby leaving the alkaline salts free to function in later relaxations.

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(67) Weizsäcker: Journ. Physiol., 1914, xlviii, 396.
(68) Weizsäcker: Sitzungsb. der Heidelberger Akad. d. Wissenschaften, B, 1917.

PHYSIOLOGICAL REVIEWS

VOL. II JULY, 1922 No. 3

THE RELATION OF THE ADRENALS TO THE CIRCULATION[1]

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The relation of the adrenal glands to the circulation presents four major questions. How is the circulation affected by adrenal deficiency and by the administration of adrenal extracts? Do the adrenal glands at any time produce sufficient adrenin to affect the circulation? If so, what factors condition adrenin discharge?

That extensive destruction of suprarenal tissue leads to marked circulatory asthenia has been recognized since the work of Addison and of Brown-Séquard. Profound asthenia leading to death invariably follows complete destruction of all the adrenal tissue. The causation of the cardio-vascular collapse has been studied by many investigators but is still undetermined. Until the epoch making work of Oliver and Schafer in 1896 it was believed that adrenal destruction results in a fatal accumulation of toxic products in the blood; without ever having been conclusively disproved, this theory has largely dropped from view because of lack of satisfactory evidence in its support.

When, however, the remarkable power of adrenal extract as a cardiovascular stimulant was discovered, it was immediately assumed and for years generally believed that the essential factor in the symptomatology of adrenal deficiency is deprivation of the so-called active principle, now widely known as adrenalin. It was assumed that this substance is constantly discharged in sufficient quantity to maintain a stimulating influence on the sympathetic nervous system. Blood pressure, which changes quickly under the influence of adrenin, has been used largely as a criterion of sympathetic stimulation.

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[1] The literature now includes hundreds of papers dealing directly or indirectly with the relation of the adrenal glands to the circulation. Spacial limitations obviously preclude specific consideration of most of these. More detailed consideration of much of this literature may be found in the new editions of Biedl's and of Vincent's well known monographs and in *Internal Secretions and Metabolism*, edited by Barker, Hoskins and Mosenthal, all of which will supposedly be available by the time this article is in print.

EFFECTS OF ADRENAL DEFICIENCY. The precise circulatory effect of adrenal deficiency was first studied by Strehl and Weiss (1). Their method was to register changes of arterial pressure ensuing upon occlusion of the lumbo-adrenal vein in the rabbit. They noted most commonly a brief fall, which returned to normal upon releasing the vein. Their results were not consistent and are not convincing in that the period of occlusion was very brief and that the depressor effects of sensory stimulation in the adrenal region were not recognized. Young and Lehman (2), in Vincent's laboratory, repeated the experiments on dogs, leaving the ligatures in place from 10 to 30 minutes. But very slight fall of blood pressure occurred and that very gradually. Young (3) independently repeated the experiment and observed no significant fall of pressure for hours. Biedl (4) reported that in case of the adrenals transposed to an extraperitoneal situs, extirpation led to a fall of blood pressure that lasted 15 to 30 minutes, but the pressure then returned to normal and remained so for 2 or 3 days.

In 1912 Hoskins and McClure (5) repeated the ligation experiments on dogs. Occluding ligatures were placed across the mouths of the lumbo-adrenal veins and, after waiting for the immediate effects of the operation to disappear, the results of closing and releasing the veins were recorded. Each experiment continued from 10 to 30 minutes. In only one case was any significant fall of blood pressure observed. Vincent and his students (6) have recently restudied the problem by comparing, over a period of from 12 to 40 hours, the course of blood pressure of anesthetized dogs with adrenals intact and those with the glands extirpated. No significant differences in blood pressure changes were noted after adrenal ligation. Bazett (7) has also found in case of cats and rabbits that adrenal extirpation fails to produce prompt fall of blood pressure. Since injected adrenin has but an evanescent effect, such observations indicate that the symptoms of adrenal deficiency are not due to lack of adrenin. That adrenal extirpation, however, results in no early significant alteration cannot, in the light of recent observations, be maintained. Winkin (8) has shown that the blood pressure reactions to cerebral anemia are modified early after occlusion of the adrenal vessels.

As to the cause of collapse following adrenal extirpation, Elliott (9) proposed a theory that minute amounts of circulating adrenin are necessary to the functioning of the sympathetic system; that is, that, without actually stimulating, they render it capable of transmitting impulses. This possibility was put to experimental test by Hoskins and Rowley (10). In a series of dogs, either normal or deprived of their adrenals, the effects of slow infusions of dilute "adrenalin" were studied. At no time was satisfactory evidence obtained that augmented adrenin in any way facilitated the transmission of vasomotor impulses. The condition of the vasomotor mechanism was studied by peripheral faradization of sciatic and splanchnic nerves and by injections of nicotin and "adrenalin." In numerous cases the adrenin infusion lessened the vasomotor irritability—sometimes to a marked degree. This was true of both the pressor and depressor mechanisms. The asthenia of adrenal deprivation does not depend, therefore, upon the failure of the sympathetic as a conducting mechanism. Neither does it depend upon a loss of sympathetic irritability, either central or peripheral, as was shown (11) by a study on a series of animals deprived of their adrenals. At a time when the animals exhibited marked weakness, cardiac and skeletal, reflex hypertension from central stimulation of the crural nerve persisted as did pressor reactions to adrenin. The reactions to nicotin, which is a selective sympathetic stimulant, were often even exaggerated. The irritability of the inhibitory fibers to the gut persisted even longer than that of the pressor fibers. That some part of the circulatory mechanism is early impaired, however, is indicated by the recent work of Rich (12), who found that hypotension follows double epinephrectomy within a period of from 4 to 7 hours, at a time when muscular strength is still retained.

That adrenin loss, itself, ordinarily plays any significant rôle in adrenal deficiency is further rendered doubtful by the fact that extirpation of one gland and denervation of the other has been shown by Stewart and Rogoff (13) to result in no apparent difference in the health of the animal, even though the adrenin output was reduced below detectable limits in the efferent adrenal vessels, hence, in the general blood stream, far below the quantity necessary to any known pharmacodynamic reaction. That adrenal denervation leaves an animal handicapped in meeting the strain of severe muscular exertion, however, has recently been shown by Hartman, Waite and Powell (14).

On the whole, it is doubtful whether the striking hypotensive effects of adrenal deficiency have their origin within the sympathetic system. Recent work of Sandiford (15) and others showing the marked effect of adrenin on basal metabolism suggests that the fault, if connected in any way with adrenin deficiency, lies in the effector rather than the conductor mechanism.

THE EFFECTS OF ADRENIN ADMINISTRATION. The outstanding results of intravenous injections of adrenal extract were accurately observed by Oliver and Schafer. In short, they noted that blood pressure is strikingly augmented, the heart stimulated and the blood driven from the splanchnic organs. As to what became of the blood thus shifted from the viscera, their original reports gave little evidence. This mass shifting seems now, however, to be the most significant feature of adrenin hemodynamics. Hartman (16) was the first to study carefully this problem, as such. His general plan of procedure was to administer standard doses of adrenin to animals in three conditions: *a*, with circulation intact; *b*, with the major splanchnic vessels ligated; and *c*, with the major extrasplanchnic vessels ligated. It was found in general that when the blood traversed the extrasplanchnic circuit, depressor effects, hence vasodilatation, resulted from the administration of adrenin; whereas when the circulation was confined predominately to the splanchnic area, the standard dose caused a rise of pressure, hence vasoconstriction.

Numerous other studies of the detailed effects of adrenin in the splanchnic area have been made by plethysmography, venous outflow and Stromuhr methods. The literature on this point has recently been summarized elsewhere (17). Suffice it to say that later work in general has confirmed the observations of Oliver and Schafer to the effect that pressor doses of adrenin result in depletion of the splanchnic domain.

Hartman's studies left undetermined the problem as to what particular structures receive the blood deflected from the splanchnic area. Since the volume of the central nervous system and of the bones is essentially fixed, and since the glandular structures in the extrasplanchnic areas are of relatively slight volume, the mass shifting of the blood must involve predominately the muscle, the skin, or both. Earlier observers had noted that ordinarily the limbs react by contraction; now and again, however, expansion, which was regarded as a passive effect, was seen.

The differential effects of adrenin in the skin and muscle were studied in the writer's laboratory (18). In dogs, studies on the volume of intact limbs and on carotid blood pressure were made simultaneously. Both pressor and depressor doses of adrenin were administered by vein. In most cases the volume of the limb decreased, irrespective of the systemic reaction. In such preparations skin and bone constitute a large proportion of the tissue mass. The effect of disarticulating the limb at the tibio-tarsal joint and removing the paw and the skin from the plethysmograph was striking. In all cases except when massive doses of adrenin were injected clean-cut expansion now resulted. The studies were extended by noting simultaneously the rate of outflow from a muscular and a cutaneous branch of the same vein. Within all ranges of dosage that could be regarded as physiological, whether pressor or depressor, increased outflow from the muscular branches and decreased outflow from the cutaneous branches were seen. These observations were subsequently confirmed by Hartman and Fraser (19) and by Gruber (20). Gruber further found that cutting the nerve to the muscle prevented vasodilatation during the earlier period in which local vasomotor tonus was abolished, but as tonus was regained dilatation could again be evoked. The outstanding effect of adrenin within physiologic limits, then, is to shift the blood from the splanchnic area and the skin to the skeletal muscles. This effect is seen irrespective of whether pressor or depressor doses are used, hence cannot be regarded as passive.

Vasodilator effects of adrenin. That adrenal extract may produce a fall in blood pressure was reported by Moore and Purinton (21) in 1900. Aqueous extracts were made from both the cortex and the medulla of the glands. The protein components were largely removed by boiling in a slightly acid medium. The experiments were carried out on 7 dogs under chloroform anesthesia. The vagi were paralyzed by atropin. It was found that intravenous injections of medullary extracts in doses equivalent to 0.005 to 0.010 mgm. of gland substance per kilogram body weight gave pressor reactions of from 20 to 40 mm. Hg. With doses below 0.001 to 0.003 mgm. only depressor effects were ordinarily obtained. Two animals, however, gave only pressor responses. These investigators left open the question whether the change from pressor to depressor reactions was due to the presence of an unrecognized impurity or to the activation of some depressor mechanism.

That adrenal extracts are able to produce vasodilatation and thus a fall of blood pressure was reported by Dale in 1906 (22). This investigator had noted that ergotoxin paralyzes sympathetic fibers that have a stimulating function, leaving more or less intact those having an inhibitory function. A dose of adrenin that, in a normal animal, would evoke rise of pressure was found, after ergotoxin poisoning, to cause a fall. Elliott (23) reported that he had occasionally observed depressor effects from very dilute solutions of adrenin, but apparently was unwilling to accept the observations as valid. Despite such observations, however, for many years the depressor effect of adrenin was regarded as negligible or non-existent. In 1912 Hoskins and McClure (5), and a year later Cannon and Lyman (24), studied more carefully the systemic reactions to adrenin in different doses. With very slight quantities it was found that the outstanding effect was depression; then as the dosage increased the depressor was gradually superseded by a pressor effect. Cannon and Lyman noted that both the pressor and depressor effects were, within limits, cumulative. They observed, also, that when the initial blood pressure was low, only pressor effects could be elicited. These results were confirmed by Hartman in 1915.

Hartman (25) and his collaborators have more recently published extensive observations on the adrenin vasodilator mechanism. They noted that if the splanchnic nerves were cut, adrenin in doses that had previously caused a rise of pressure now caused only a fall. The part played by the central nervous system in the vasodilator reaction was also studied. A segment of intestine or a limb was cut off from the body circulation and independently perfused with warm oxygenated Ringer's solution. The part was connected with the rest of the body only by its nerves. The organ was then placed in a plethysmograph or the venous outflow was measured directly. Adrenin injection into the general circulation brought about vasodilator effects in the isolated organ, and these must have been mediated by the central nervous system or ganglia. Decerebration failed to affect the reaction. When the medulla was destroyed the systemic depressor reaction was converted to pressor, but the dilator mechanism in both the limb and the intestine was found to be still functional. Destroying the spinal cord failed to abolish the dilator response, hence it was concluded that the effect was partially determined outside the central nervous system, proper. Subsequent investigation showed that local vasodilator effects could be abolished by the destruction of the sympathetic or the dorsal root ganglia. It was found possible, on the other hand, to bring about dilatation by the application of adrenin directly to these ganglia. A systematic study was made of the occurrence of the vasodilator reaction in various species of animals. It was found absent in reptiles, birds and rodentia (rats and rabbits), but present in the opossum; it was demonstrated in the carnivora (cats, dogs and ferrets). But in the cat the mechanism was not functional until about the 11th week of life.

Factors that modify adrenin reactions. Various factors have been found to modify the systemic reaction to adrenin. The Goetsch test for exophthalmic goiter depends upon the augmented sensitivity of the sympathetic system with resulting increase in the response to the drug. Parathyroid extirpation, with its augmented sympathetic irritability, has a similar effect. Simonds (26) found that in dogs subjected to anaphylactic shock and peptone poisoning, the response to adrenin was either much diminished or entirely lost. Schiff and Epstein (27) have reported that general debility in children markedly decreased the reaction to adrenin. Collip (28) found that the pressor reaction was augmented and prolonged by the administration of tissue extracts, whereas the depressor reaction was lost or even converted to a pressor reaction. Similarly, he has shown in a striking way that under identical conditions a given dose of adrenin will cause a depressor response in a dog under light anesthesia, whereas increasing the depth of the anesthesia results in a pressor reaction to the same dose. As is well known, if the anesthesia is made very deep, the pressor effect is finally diminished (29). Cocain somewhat sensitizes the dog to adrenin. Hartman found that a given dose of adrenin which evoked constriction in a limb brought about dilatation when artificial heat was applied. The effects of hemorrhage are sufficiently indicated in Cannon and Lyman's observations that any factor leading to low initial tension converts the depressor to a pressor reaction. Snyder and Campbell (30) perfused adrenalin through the circulation of frogs. It was found that decreasing the acidity of the menstruum increased the vasoconstrictor effect; and vice versa. Collip (31) extended these observations to the dog. He found that the sudden administration by vein of a fairly large dose of sodium carbonate resulted in a change of depressor reactions to pressor. A subsequent injection of acid sodium phosphate was found to convert the pressor to a depressor effect. Such reversals could be obtained repeatedly in the same animal.

From the foregoing it is obvious that there is no justification for classifying adrenin as a vasoconstrictor agent. It is either vasodilator or vasoconstrictor, depending upon the amount used, the organ affected and the conditions of the experiment, as regards body temperature, depth of anesthesia, action of such deleterious factors as tissue extracts, anaphylaxis, etc. A failure to recognize the importance of these modifying factors accounts in part, perhaps, for the existing conflicts in the evidence regarding adrenal pharmacology.

Effects of adrenin on the heart. As regards the isolated heart the evidence is strikingly consistent that adrenin causes stimulation. Burridge (32) has shown that a slight amount of adrenin markedly improves the activity of a heart exposed to unbalanced Ringer's solutions. He believes that the adrenin effect is particularly related to the calcium ion effect. With the heart in situ and the vagi intact, the resulting high blood pressure, when larger doses of adrenin are administered, usually leads to secondary depression,—at least of the chronotropic function. On teleological grounds dilatation of the coronary vessels would be expected, and in most animals such results have been obtained. In man, however, Barbour noted that rings cut from the coronary arteries responded to adrenin by contraction only. The problem was further investigated by Barbour and Prince (33), using another primate, the Macaccus rhesus, the experiments being controlled with rabbits. Isolated hearts were perfused with diluted blood to which hirudin was added. In the rabbits increased coronary outflow always resulted, while in monkeys precisely the opposite effect was seen under all conditions of high or low perfusion pressure, with beating or resting hearts and with all effective doses of adrenin. The doses reported, however, were grossly in excess of any to which the heart of a normal animal is supposedly ever subjected. The problem is in need of further study with regard to the accessory conditions known to influence adrenin reactions and especially with regard to dosage.

Effects of adrenin upon pulmonary circulation. The effects of adrenin upon the circulation of the lung have been studied by a number of careful investigators, beginning with Brody and Dixon and Plumier in 1904. The earlier observers ordinarily used quantities at least hundreds or thousands of times as great as those in the normal blood stream. In general, under such conditions, increased pulmonary pressure and vasoconstriction were seen. Tribe (34) perfused the lungs of numerous cats, dogs, rabbits, guinea pigs and ferrets with adrenin in defibrinated blood. With high dilutions, dilatation was obtained, while with large doses, constriction resulted. Schafer and Lim (35) have reviewed the evidence and added many observations of their own. They employed adrenin in dilutions ranging from 1:2,000 to 1:62,000. In nearly every case constriction resulted.

Studies of the effects of epinephrin on the pulmonary circulation in living animals have given varied results. Some observers have reported increased pressure and flow, and others, decreased. Desbouis and Langlois (36) using an electrical method determined the rate of the blood flow in the lungs of the dog. Doses of 0.05 or 1.025 mgm. accelerated the flow; with larger doses retardation was seen. Anderes and Cloetta (37) came to the conclusion that adrenin ordinarily has no effect upon pulmonary circulation. Schafer and Lim (35) studied the simultaneous effects on pulmonary and systemic blood pressure. The results varied somewhat in different animals, but the effects were more or less parallel in the two systems. The sum total of the available evidence indicates that the physiologic effects of adrenin in the lungs are not such as significantly to influence the systemic circulation.

Effects of adrenin on venous pressure. Venous pressure, according to most observers, is not greatly affected by adrenin, except in large doses that result in back pressure from the heart. Connet (38) has recently reviewed the literature and reported a somewhat extensive series of experiments upon 50 dogs and 25 cats. She worked always with quantities of adrenin sufficient to cause a rise in arterial pressure. In order to eliminate the effects of respiratory movements and of anesthesia, certain experiments were carried out on curarized and decerebrated animals. In these, such doses as 0.15 mgm. caused marked increase in arterial pressure, leaving pressure in the vena cava unchanged. Ordinarily, however, some increase in the venous pressure was noted, irrespective of whether the heart rate was increased or decreased. Altogether, it would appear that adrenin has relatively little effect upon the veins and this, such as to facilitate the systemic circulation.

From the foregoing data it appears that the adrenals have an important relation to the circulation and it is at least possible that adrenin itself plays a significant rôle. As to whether such is the case under ordinary circumstances, opinion is divided. Some students still tend to the opinion that hypotension resulting from adrenal destruction is due merely to loss of circulating adrenin. Direct attempts to prove this have for the most part given negative results.

Is ADRENIN NORMALLY SECRETED IN SIGNIFICANT QUANTITY? The ideal method of determining whether adrenin is normally secreted in sufficient amount to affect the circulation would be to ascertain the minimal amount that would exert a detectable influence and then to determine whether, under normal conditions, the adrenal glands, together with accessory chromophil tissue, secrete this amount. Up to the present time, however, determinations of the adrenin output under normal conditions have not been made. It would seem that such could be approximately achieved by utilizing Biedl's technique of transplanting the glands to a subcutaneous locus, with care to preserve intact both the circulation and innervation and, later, securing and assaying the efferent blood. With proper allowance for the dilution factor, the amount of adrenin in the blood stream could then be calculated.

The nearest direct approach to the problem has been made by Trendelenberg (39), who withdrew blood from the carotid of the rabbit and passed it through the circulatory channels of the frog. His results indicate that adrenin occurs in the arterial stream at most in a dilution of 1 to 2 or 3 billions. This is a quantity that has no perceptible influence in mammals. Moreover, it was not demonstrated that the minute amount of pressor substance found by Trendelenberg was actually adrenin. This experiment obviously left undetermined whether secreted epinephrin exerts an appreciable influence in the lesser circulation or in the respiratory processes, intervening between the adrenals and the carotid artery.

Observers of a decade ago were of the opinion that adrenin is secreted in fairly high concentrations. Later investigators, however, have found the concentration much less. The method employed in such determinations has been ordinarily to open the body cavity and draw off the blood from the lumbo-adrenal veins, either directly or into a pocket fashioned from the vena cava and assaying it outside the body. Using this method, Stewart and Rogoff (40) and Hoskins and McClure (5) noted in dogs a total output approximating 0.0002 mgm. per kilogram body weight per minute. More recently, Stewart and Rogoff (41) have made a few determinations in dogs, obtaining the blood more directly through a lumbar incision. They secured an output approximately the same as with the older technique. However, when assayed by the pupillary reaction of the experimental animal the output in cats appeared to be about 0.0007 mgm. (42). It is to be noted that in all such cases two factors which probably more or less vitiate the results have been introduced, namely, anesthesia and trauma. The quantity of adrenin necessary to affect blood pressure was found by Hoskins and McClure (5) to be about 0.0005 mgm. per kilogram per minute. Approximately five times as much was required for minimal pressor effects. The determinations of the threshold have been made for the most part in animals under anesthesia, but work in the writer's laboratory, in which the reactions to adrenin were determined under local and under general anesthesia, showed that the thresholds do not vary significantly unless the anesthetic be deep. The data thus obtained indicate roughly that the amount of adrenin secreted is of the general magnitude of that requisite to influence circulation, but the experimental methods have involved too many uncontrolled variables to be convincing.

A number of indirect attacks upon the problem has been made. If secreted adrenin were a normal factor in regulating the circulation it would presumably have a "sustaining" effect. If it be assumed that blood pressure is being continuously sustained by adrenin discharge, the addition of a slightly greater quantity of the drug should result in corresponding increase of pressure. If, however, a dog of quiet disposition be carefully anesthetized with minimal excitement and with minimal trauma and an artery and vein be cannulated, small quantities of adrenin can be introduced into the blood stream with absolutely no effect upon the blood pressure. This observation in itself indicates that no significant amount is being secreted, since an existing threshold has to be passed. As the quantity of injected adrenin is slowly increased, the first reaction to appear is depression. If the infusion continues at a constantly increasing rate, ultimately the depressor effect disappears, to be followed by a pressor effect. The logical conclusion from such observations is that the adrenals are ordinarily quiescent and that the first effective result of adrenin discharge is decrease of arterial pressure, brought about supposedly by dilatation in the skeletal muscle.

A somewhat less convincing experiment is to determine simultaneously the threshold for adrenin vasomotor effects and peristalsis-inhibiting effects. In the writer's experience, peristalsis is brought to a standstill with considerably higher dilutions than are required to exert an effect on circulation. Experiments along this line have been relatively few, however, and have extended over a period of but a few minutes. It is conceivable that adaptation might later occur and intestinal peristalsis be resumed, even while adrenin in pressor quantities was being circulated.

The evidence as a whole militates against the supposition that blood pressure is ordinarily maintained by the stimulating influence of small quantities of adrenin continuously secreted. It is, of course, obvious that all the experiments cited have been made under artificial conditions and hence do not exclude the possibility that in the normal animal, under perfectly normal conditions, such a minimal pressor influence may be exerted but, in the face of the evidence, the burden of proof is upon those who hold this view. A considerable number of clinicians has consistently maintained the view as supporting the theoretical etiology of so-called "hypoadrenalemia." In this connection, it may be recalled that Cannon and Lyman and Collip have reported that with conditions of low blood pressure adrenin in any effective amount exerts a pressor influence. The clinical theory mentioned, if worked out with sufficient ingenuity, is not, therefore, untenable, although distinctly improbable. The more probable hypothesis to account for the low blood pressure which follows adrenal destruction is that not the medulla but the cortex is at fault. Such a hypothesis must be invoked to account for the final death of an epinephrectomized animal, for it is impossible to maintain life by the artificial administration of adrenin in any way or in any quantity.

FACTORS CONDITIONING ADRENIN DISCHARGE. However unlikely it is that adrenal discharge exerts a significant effect upon the circulation under ordinary conditions of quiescent existence, there has accumulated much evidence that under special conditions the rate of discharge may rise to an effective level. The problem has been under almost continuous investigation in the laboratories of Stewart and Cannon for more than a decade and a large proportion of the available data has come from these two sources.

Nervous control of adrenal discharge. That stimulation of the splanchnic fibers running to the adrenal glands may lead to augmented adrenin discharge was first observed by Biedl and by Dreyer, who noted that blood collected from the adrenal veins during splanchnic stimulation had a greater pressor effect than that obtained without such stimulation. Recently Tournade and Chabrol (43) have confirmed this by a crossed circulation experiment in which the adrenal blood from one dog was shunted directly to the jugular vein of a second. Splanchnic nerve stimulation in the first gave a rise of blood pressure in the second. Stewart and Rogoff have further shown that section of the splanchnic nerves leads to a marked depression of adrenin output—at least under the artificial conditions of their experiment. The demonstration of functional nerve fibers to an organ is essentially tantamount to proof of its central control.

Langley found that in the cat the splanchnic nerves received white rami from the fourth or fifth thoracic segment to the second lumbar segment of the spinal cord. The immediate source of fibers to the adrenals would, therefore, fall somewhere within these limits. Stewart and Rogoff (44) attempted to locate more precisely the region of outflow by sectioning the spinal cord at various levels. They investigated the adrenal output by the denervated eye reaction of Meltzer and the intestine-uterus method of Stewart. They were unable to detect any difference of adrenin secretion following destruction of any part of the central nervous system as low as the last cervical section. When, however, the cord was further destroyed it was found that secretion ceased and it was concluded that the fibers concerned in the liberation of adrenin do not extend much below the third thoracic segment. They concluded that there is a center for adrenal discharge in the upper part of the thoracic cord. They were unable, however, to demonstrate activation of this center except by pharmacologic means (strychnia or sodium carbonate).

Cannon and Rapport (45) have investigated further the location of the adrenal center. They used as criterion of adrenal discharge, change of the rate of the denervated heart. Careful precautions were observed to eliminate incidental variables such as redistribution of the blood, differences in initial blood pressure, changes in respiration, etc. That the adrenal glands were secreting under the conditions of the experiments was indicated by a decrease in the heart rate when the adrenal glands were removed. It was found possible to bring about an increased heart rate, varying from 12 to 40 beats per minute in various instances, by stimulation of the sciatic or brachial nerves. Successive ablations of the higher portions of the brain failed to abolish this accelerating effect until transection was made about 2 mm. caudad to the corpora quadrigemina. When, however, this portion of the central nervous system was destroyed, sciatic or brachial stimulation no longer evoked increased heart rate. It had previously been shown that removal of the adrenal glands under similar experimental conditions likewise abolished the heart acceleration; hence, it was concluded that the center for adrenal discharge lies in the region mentioned. It was found further that stimulation of the central end of the vagus or of the depressor nerve caused a slowing of the denervated heart by as much as 24 beats per minute. This reaction likewise persisted after ablation of the brain as low as the corpora quadrigemina, but disappeared when the brain stem was destroyed a few millimeters caudad to the corpora. This depression likewise disappeared when the adrenal glands were removed.

Methods of accelerating adrenal discharge. The conditions that determine adrenal activity have been under careful study for more than 10 years. As was early recognized, the presence of sympathetic secretory nerves to the glands establishes strong a priori likelihood that adrenal secretion would be brought about by any influence which activates the sympathetic system generally; that is, by such conditions as cause stoppage of gastro-intestinal peristalsis, increased blood pressure, dilatation of the pupils, contraction of the pilo-motor muscles, etc. It has long been known that such reactions are readily evoked by strong emotions, by pain, and by asphyxia. It is likewise easily demonstrable that they may be evoked by the intravenous injection of adrenin. To prove or disprove, however, to the satisfaction of critical physiologists whether adrenal discharge actually occurs under experimental conditions has presented a very difficult problem. A voluminous controversial literature on the point has accumulated. The evidence, pro and con, can be found for the most part in articles by Stewart and Rogoff and by Cannon and his collaborators. But a few of the observations can be mentioned here.

The deduction that emotions cause adrenal discharge was put to experimental test by Cannon and de la Paz (46) in 1911. By means of a flexible catheter introduced through the femoral vein into the vena cava above the level of the lumbo-adrenal veins, blood was secured from cats both before and after emotional excitation. This blood was then assayed for its adrenin content by use of strips of longitudinal intestinal muscle. Evidence was thus obtained of significant adrenal discharge during emotion. Removal of the adrenal glands abolished the reaction. By essentially the same technique, Cannon and Hoskins (47) obtained evidence of adrenal discharge during asphyxia and strong sensory stimulation. Anrep (48) shortly afterward noted that a denervated limb or kidney first expanded, then contracted sharply, when the central end of the cut sciatic nerve was stimulated. This contraction occurred in the face of an augmented blood pressure, hence could not be regarded as passive. Since the organ was denervated the contraction must have been brought about by some chemical substance. Since the reaction disappeared when the adrenals were excluded the chemical substance was supposed to have been adrenin. Anrep's observation on the denervated limb was confirmed by Pearlman and Vincent (49). These phenomena Stewart and Rogoff undertook to explain on a basis of redistribution of blood or changes in the rate of flow. Burton-Opitz (50), however, as well as other investigators, have recorded evidence indicating that such explanations are not tenable. Gley and Quinquaud (51) studied the effects of asphyxia on adrenal discharge. In brief, they noted that 4 to 8 cc. of adrenal blood caused as great rise of blood pressure when obtained during asphyxia as did 15 cc., obtained without asphyxia. Likewise, they noted that 20 cc. of blood from the vena cava above the lumbo-adrenal veins, after 3 or 4 minutes of asphyxia, caused a rise of arterial pressure from 24 to 45 mm. higher than was produced by the same quantity of caval blood similarly obtained before asphyxia. Similarly Kellaway (52), using the pupillary reaction, obtained data indicating that asphyxia causes adrenal discharge. More recently Gley has taken the ground that the adrenals do not play a significant part in the experimental vasomotor reactions.

An important advance in technique was introduced by Cannon (53) in 1917. This was the use of the denervated heart as an indicator of adrenal discharge. Stewart had previously used the denervated pupil, but changes in pupillary diameter lend themselves less readily than do changes of heart rate to objective recording. Cannon and Rapport (54) showed that in an animal in which redistribution of the blood was largely eliminated by tying the carotid, brachial and renal arteries and the lower aorta and by cutting the mesenteric nerves, brachial and sciatic stimulation resulted in increased heart rate, varying from 12 to 39, most of the figures falling in the upper range. Such results have been criticised by Stewart and Rogoff on the grounds that it is possible to demonstrate cardiac acceleration, even with the adrenals removed. The explanation of the discrepancy apparently lies in the fact that in animals that are digesting meat there may be discharged from the liver an unknown substance which stimulates the heart (55). If, however, the hepatic nerves are severed, this no longer enters as a disturbing factor, and in such animals clean-cut acceleration of the pulse can be obtained by sensory nerve stimulation and subsequently done away with by the removal of the adrenal glands. That these effects were due essentially to adrenal discharge rather than to circulatory changes of any sort was shown by the injection of adrenalin at a constant rate, simulating normal adrenal discharge, while the sensory nerves were being stimulated. In such cases the augmentation of the heart rate failed to appear; hence it cannot be argued that the tachycardia was due to any sort of redistribution of a constant amount of adrenin coming from the glands.

Recently Cannon and Carrasco-Formiguera (56) have restudied the problem, using Stewart's original technique except that the denervated heart, instead of the denervated pupil, served as test object. They found that when the blood from the adrenal glands was restricted from the circulation, reflex cardio-acceleration was prevented. After removal of the venous block the response again appeared. The time interval between the beginning of reflex stimulation and the cardiac response was approximately the same as that seen after stimulating the splanchnic nerve or injecting adrenin by vein. Similarly, asphyxia for 45 seconds resulted in cardio-acceleration when adrenal egress was possible but failed to do so when the adrenal discharge was blocked.

Altogether, it seems that, despite the ingenuity with which the data have been criticised, enough careful and concordant observations are now available to justify the conclusion that the adrenal glands are controlled by the central nervous system and that they are stimulated to effective secretion, as would be expected, by such influences as affect other organs under sympathetic control. These influences are, especially, strong emotions, pain and asphyxia. The adrenin thus discharged reinforces the sympathetic stimulation leading to a mass shifting of the blood from the "vegetative" organs to those involved in neuromuscular exertion.

RÉSUMÉ

The adrenal glands have a definite pharmacologic relation to the circulation by virtue of their production of adrenin. Adrenin causes stimulation of the heart, vasoconstriction in the splanchnic and cutaneous regions and dilatation in the skeletal muscle. Increased or decreased blood pressure may result depending upon dosage and various accessory factors. Adrenal extirpation with its resulting circulatory collapse proves the existence of a physiologic relationship also. This collapse is not entirely if at all due to adrenin lack since it cannot be long forestalled by administration of the drug and it does not ensue when adrenin secretion is reduced below detectable limits. The slow development of the symptoms of epinephrectomy also indicates that they are not due to sudden failure of adrenin as a stimulatory substance. Direct experimentation shows that adrenin often depresses sympathetic irritability. Marked symptomatology develops while the sympathetic system responds well to stimulation. If adrenin deficiency is a factor, it probably operates in the effector rather than the conductor mechanisms. The adrenals are stimulated to secretion by splanchnic nerve irritation, hence are supposedly under central control. Evidence of the existence of an adrenal center immediately caudad to the corpora quadrigemina has been reported. The preponderance of the evidence indicates that adrenal depression is evoked by stimulation of the vagus or depressor nerves and augmentation by asphyxia, pain and emotional excitement. This augmentation results in a mass shifting of the blood from the skin and viscera to the organs involved in neuromuscular exertion. The cortex is probably the indispensable part of the adrenal. The medulla apparently serves merely to reinforce the sympathetic system in times of stress.

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THE INTERPRETATION OF SPINAL REFLEXES IN TERMS OF PRESENT KNOWLEDGE OF NERVE CONDUCTION

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The contrast between the phenomena of conduction in the peripheral nerve trunk and in the reflex arc has been the theme of much discussion in the literature on the nervous system. The comparative simplicity and approximately machine-like regularity of the response to stimulation in the nerve trunk, as compared with the great complexity of behavior in the animal whose central nervous system is intact, has led to the general view that in the structures of the central gray matter exist functional capacities differing fundamentally from those of the nerve fiber, and indeed having no counterpart in so simple a mechanism as the nerve-muscle preparation. In these obscure functional capacities of the gray matter are supposed to lie the secret not merely of the coördination and adaptation of spinal reflexes, but of volition, memory and habit-formation.

Faced by the vast complexity of the entire central nervous system in even the lowest of vertebrates, we have sought to attack the problems of its physiology by looking for the key to these functions in the spinal reflexes, taking the spinal cord as the simplest mechanism involving the gray matter.

The contrast between nerve-trunk and reflex arc may be viewed from the angle of the diversity of purpose which the structures serve. The nerve fiber apparently exists for the purpose of transmitting messages to remote parts, rapidly, economically and without modification. The central structure appears to serve as a junctional point where messages from many regions may be correlated, relayed and distributed to other regions. In this respect the fibers and centers may be likened to the wires and central offices, respectively, of a telephone system.

Anatomically the nerve fiber is characterized by its comparative simplicity, extending as it does for great distances without branching or changing much in size or form. In contrast with this the gray matter presents a picture of the most prodigious complexity, with intricate end branches and dendrites providing the connections for extensive coördination of conducting paths (1, section II).

Finally, the contrast between nerve-trunk and reflex arc may be viewed from the angle of experimentally observed functional differences. Sherrington in 1906 (2, p. 14) summarized the differences between conduction in nerve-trunk and reflex arc, the most important of which are as follows:—Reflex conduction shows *a*, slower speed as judged by latency of response; *b*, after-discharge, i.e., persistence of response after stimulation has ceased, often for several seconds; *c*, summation, single stimuli in many reflexes failing to produce any response, whereas a repeated series is effective; *d*, irreversibility, conduction from afferent to efferent neurones being possible, but in the reverse direction through the central structure, impossible; *e*, fatigue on continued stimulation, in contrast with the nerve-trunk which exhibits extraordinary resistance to fatigue; *f*, far greater variability of threshold, or ease with which responses can be evoked; *g*, mutual relations between allied or antagonistic reflex arcs, manifesting themselves as reinforcement or inhibition; *h*, far greater dependence on blood supply and oxygen, and correspondingly greater susceptibility to anesthetics. Broadly speaking, these are the most notable differences between the reflex arc and the nerve fiber as regards conduction.

We are confronted with the question; wherein lies the basis for these differences? As already indicated, microscopic anatomy reveals great structural differences; the nerve fiber presents a cylindrical structure surrounded by a membrane, usually by a sheath of myelin, separating the protoplasm within from the body fluids without; the reflex arc, on the other hand, comprises besides relatively simple axons conducting to and from the central structures, an intricate series of end branches whereby the afferent neurone makes contact with the central and motor neurones, elaborately branched dendrites converging in cell bodies containing nuclei, and points of contact or connection between the end branches and the dendrites, little understood as to their finer detail, designated synapses.

Sherrington (2, pp. 15–17) presents cogent reasons for supposing that the nerve-cell body is not the part of the reflex arc to which its peculiar conducting properties are referable, and for looking instead to the synapse, or point of connection between neurones. He suggests that a membrane or surface of separation across which conduction between neurones must occur, might readily account for the functional properties of the synapse.

Loeb (3, pp. 4–6), some years earlier, emphasized the continuity of the conducting protoplasmic path as the essential feature in the reflex arc, a view which tends to place the burden of its properties rather on the distribution of the pathways than on the presence in them of a transverse membrane of separation between cells.

We might conclude that the indisputable difference between the reflex arc and the nerve-trunk lies in the intricately branching system of interconnections on the one hand, and the isolated, unbranched arrangement on the other. Other significant structural differences are more open to question. In particular, whether there is indeed a true surface of separation between connecting neurones, or whether there is actual protoplasmic continuity, seems not to be settled as yet by any histological research, although the recent work of Marui (4) points to the latter view. So far as I am aware, no thorough attempt has been made to see if other possible attributes of the synapse might provide a basis for its behavior in as satisfactory a manner as the surface of separation to which Sherrington has drawn attention. I shall return to this consideration later.

Most of the literature concerning the physiology of the central nervous system was written before the present concepts of nerve function had been developed. For this reason we find the conducting paths treated as if they were pipe lines through which continuous streams of an imaginary fluid could be poured. Correspondingly, stimulation, as treated in the literature, might be likened to opening a valve or faucet whereby any desired quantity of this fluid is poured into the stimulated nerve. The application of an interrupted current was assumed to start a steady flow of nervous energy along the stimulated nerve, the flow being graded in volume according to the strength of the stimulating current. Then, as the resulting stream of nervous energy encountered various resistances in its path through the central connections, it was supposed to lose its strength little by little in proportion to the resistance encountered (2, p. 156). Little or no attention was paid to the individual nerve impulses making up the stream. The word "excitability" was used in a loose sense without definition, variations in the threshold of stimulus and in the magnitude of response being used indiscriminately as criteria, as if they necessarily had the same meaning. It was natural for physiologists to treat nervous activity in this way, since the apparent gradation of response to stimuli of various strengths, both in the nerve-muscle preparation and in the spinal frog, suggested that the strength of a nerve impulse might be graded as easily as the current flowing in a wire.

Researches in the last fifteen years, especially those of Lucas and Adrian(5), (6), (7), (8), (9), (10), (11), (12) have thrown a flood of new light on the physiology of the excitable tissues (nerve and muscle), which must necessarily modify our interpretation of the observations, past and future, on the activities of the central nervous system. Specifically there is need of revising all statements which imply without qualification that this system functions by means of continous streams of nerve energy which may be varied in the manner of streams of water, at least wherever axons are concerned. Also, as Lucas has pointed out (11, p. 48), it behooves us to state what we mean more explicitly than in the past when we speak of excitability.

Even since the firm establishment of the modern concepts concerning the nerve impulse, very few papers dealing with the central nervous system have shown the heed for them which an adequate treatment of the subject demanded. Notable exceptions are Sherrington's recent papers (13), (14), (15) on the flexion reflex and Martin's paper (16) on the vasomotor reflexes, both of these authors having given the most careful consideration to the implications of the new knowledge. Not only do these modern concepts of nerve function place definite restriction upon the events we may suppose to occur in the various axons of the nervous system, but they open to us a vista of great possibilities of drawing in the future a simplified picture of the working of the nervous system. It is conceivable that through a great generalization in this direction, much that is now regarded as quite inaccessible to analysis will become intelligible in relatively simple terms.

The exact nature of the nerve impulse is not yet understood, but such a definite picture of its general nature has emerged from the abovementioned researches that we may already begin to make far-reaching changes in our interpretation of the nervous system as a whole. The analysis of the functional response in excitable tissues has been carried far enough to indicate a most significant and fundamental similarity in the behavior of all those subjected to quantitative study. Cardiac and skeletal muscle, nerve and the junctional tissue between nerve and muscle, all reveal in their response to stimulation certain essential and fundamental properties in common, differing quantitatively, but not qualitatively. The disturbance which results from stimulation and its mode of initiation both seem to be essentially the same in all. That in tissues so different in structure and embryonic ancestry as those mentioned, these essential elements of function should be the same in all, is a matter of profound significance. It suggests the inherent propensity to react in this way as perhaps a basic property of living cells in general. At all events, it establishes the probability that the propensity exists in all cells in the nervous system, whose embryonic kinship to each other is far closer than their kinship to muscle.

The establishment of a common, and fairly well understood basis for the execution of the functional response in all those tissues (nervous and muscular) which have yielded to functional analysis, suggested to Lucas the possibility of its universal application, and led him to raise the following question: "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?" (11, p. 2.)

The scope of this idea is large. A reduction of the elements of neural activity underlying consciousness and behavior to the single basis of the nerve impulse, many of whose physical properties are now known, and whose true physical nature may come to be more fully understood in the future, would be a generalization comparable to the reduction of all the various chemical elements to their constitutent protons and electrons (cf. 17, p. 348).

Elementary functions of excitable tissue. Let us now summarize the salient points in the physiology of the excitable tissues as revealed by recent researches. These tissues may be excited by various classes of stimuli, mechanical, thermal, chemical and electrical; of these, electrical stimuli are unique in their ability to excite the tissues without in any way damaging them, and in the ease with which they may be regulated for quantitative study. When an electric current is passed through a portion of a nerve or muscle fiber a local change occurs which is limited to the vicinity of the region through which the current flows. If this local change does not attain a certain critical intensity it produces no remote effects whatever; if it is made sufficiently intense, however, it will set up a disturbance which is conducted away from the point of stimulation over the entire length of the fiber. A fundamental difference exists between the local change and the disturbance which is conducted away from the point of stimulation, a difference which was first elucidated by Adrian and Lucas in 1912 (18, p. 69). To the local change they gave the name "local excitatory process," to the resulting disturbance which sweeps over the tissue, the name "propagated disturbance," each designation being the most non-committal possible, expressing the most essential features of each. The local excitatory process is distinct from the exciting current, and can be shown experimentally to persist for a time after this has ceased to flow, the rate of subsidence being characteristic for each tissue (19). Its intensity can be graded, depending on the strength and duration of the exciting current. The researches of Nernst, Lapique, Lucas and Hill (20), (21), (22), (23), (24), (7), (25) all point to the view that this local excitatory process consists in a concentration of ions at some point in the tissue. The propagated disturbance which results only when the local excitatory process reaches a certain intensity, sweeps over the tissue, leaving it refractory to further stimulation for a brief interval of time. Normally it obeys the "all-or-nothing" law (9), (10), (26); that is, it cannot be varied in intensity by any variation in the strength of stimulus. The propagated disturbance has been known classically in the case of nerve as the "nerve impulse," in the case of muscle as the "wave of excitation." It is essentially the same sort of disturbance in both tissues, and the single non-committal designation of Lucas is perhaps the best name by which to recognize it wherever it occurs. Following the absolute refractory phase, there is a period of recovery, the relative refractory phase, during which the threshold of excitation is abnormally high, and the size of response which can be evoked is subnormal, both returning gradually to normal. The size of response which can be evoked at different stages during the recovery process may be measured in the case of nerve by either of two criteria, *1*, the size of the electric response; *2*, the distance the disturbance can travel without extinction through a narcotized region where conduction occurs with a "decrement" (18, p. 95). If the fluid in which the tissue is immersed is slightly acid there is, following the relative refractory phase, an apparently supernormal phase of recovery. During this supernormal phase the excitability and the magnitude of response both become slightly greater than their final resting values (18, pp. 111–114), (27). Adrian has shown (28) that neither the excitability nor the size of response becomes greater than would be found if the same tissue were in neutral fluid. The supernormality is only relative, due to the fact that in an acid medium the final resting stage is subnormal when compared with the condition of the tissue at neutrality.

Perhaps the most important feature of the conception of the nerve impulse which has come from these researches is the fact that the energy of propagation of the disturbance comes not from the stimulus, but from the fiber itself. It has been likened (11, p. 23) to the burning of a train of gunpowder, in contrast with the transmission of a sound wave whose energy comes entirely from its initiating source. This fact, now well established, should put an end to all efforts to explain the nerve impulse simply as a transient current of electricity conducted along the fiber on on the same principle as in an insulated wire; the dynamics of the two modes of conduction are fundamentally different.

The most striking item of evidence that the source of energy is the nerve fiber itself was Adrian's experiment with the interrupted areas of narcosis (9). It was known that a nerve impulse passing through a narcotized region is not extinguished at once, but by a gradual process of decrement. Adrian showed that if the impulse is in this way almost extinguished and then allowed to emerge into a normal region beyond, it recovers at once to its full magnitude. This observation will have an obvious and most important bearing on the physiology of nerve centers, if, as has been suggested, these include regions where conduction occurs with a decrement.

An important exception to the all-or-nothing principle of response in nerve has been shown to occur when the stimulus is applied in a narcotized region which is conducting with a marked degree of decrement (29), (30). Here a certain measure of gradation in the size of response may result from gradation of the strength of stimulus.

One of the most interesting and important features of the functional response in the excitable tissues is the electric response, or action current. This is well known to occur in nerve, skeletal and cardiac muscle and in glandular tissue. It consists of a wave of lowered electrical potential (with respect to inactive portions of the tissue) which sweeps along the excited fiber, marking the progress of the propagated disturbance. Aside from its theoretical interest, it serves as a convenient indicator of function in these tissues, especially in the case of nervous tissue, in which it is the only direct and well-defined objective evidence of activity; it is thus most useful in the study of the nervous system.

The most probable explanation of the various manifestations and attributes of the functional response in excitable tissues is the membrane theory as formulated for the nerve impulse by Brünings (31). This theory holds that the essential element in the structure of the fiber is a semi-permeable membrane surrounding it, which, because it is less permeable to some ions than to others, becomes polarized, i.e., establishes a difference of potential between the inside and the outside of the fiber; and that the nerve impulse consists in a transient breaking down of this polarization through increase in permeability. An electrical response, such as that just described, would inevitably result from a progressive transient break-down of polarization, and Lillie has offered good reasons for supposing that the action current thus produced is the actual mechanism whereby the disturbance is propagated along the fiber (32). He has shown (33), (34) how an iron wire with a film of oxide on its surface, immersed in nitric acid, furnishes in its behavior a striking counterpart of the nerve impulse in several respects. The analogy must not, however, be followed too far, for whereas the nerve fiber during its relative refractory phase responds with a subnormal impulse, this is conducted without decrement along the fiber (11, p. 39), but the "iron model" when stimulated during its relative refractory phase conducts with a decrement similar to that induced in a nerve by narcosis. This difference probably marks a point of fundamental divergence between the two systems. For a more detailed consideration of the membrane theory reference should be had to Lillie's review in this journal (35) and to a paper by Troland (17) in which there is a suggestive discussion of some theoretical aspects of the membrane theory, but in which the discrimination between fact and speculation is not as clearly emphasized as would be desirable.

As indicated above, certain of the most fundamental properties of the excitable response have been found to be essentially the same in nerve, skeletal and cardiac muscle and the junctional tissue of the nerve-muscle preparation. The established facts in this connection should be more explicitly enumerated. The relation between the strength and duration of an electric current just sufficing to excite the tissues was worked out by Lucas with a high degree of quantitative refinement (5). The duration of the current beyond which further prolongation ceases to reduce further the strength required to excite, is taken as the measure of the rate of subsidence of the local excitatory process. Lucas found (7, p. 245) that under like conditions this duration for different tissues in the frog was as follows:

	Seconds
Cardiac muscle (ventricle)	2.0
Sartorius muscle	0.02
Motor nerve	0.003
Neuro-muscular junctional tissue (β-substance)	0.0009

In spite of the large quantitative differences, the shapes of the curves relating minimal current strength to duration are practically the same for all these tissues. For the sake of more accurate comparisons of excitation time in different tissues, Lapique (36) has introduced a constant which he calls "chronaxie," the duration of a constant current just sufficing to excite if it has double the strength of the threshold current of unlimited duration.

The all-or-nothing law of response is classical in the case of the heart; it is virtually established for the individual skeletal muscle fiber by the work of Lucas (6) and the later work of Pratt (37); it is well established for nerve by Adrian's experiments (10). The refractory phase, which is an inseparable concomitant of the all-or-nothing type of response, has been measured in the case of cardiac and skeletal muscle and in nerve (38), (39), (40), (41), and the recovery follows the same sort of course in each case; the duration of the refractory phase differs quantitatively in these three tissues in a manner closely proportional to the differences between excitation times given above. The electric response occurs in the same three tissues, having much the same form in all; furthermore, its total duration, rate of increase to maximum and rate of decline bear nearly the same relation in the three tissues as do their refractory phases (42) and excitation times (chronaxies).

Thus far the evidence points strongly to a community of functional propensity in these tissues, the greatest difference between them being one of time relations. So far as I am aware there has been no proof of all-or-nothing response, refractory phase or action current in the neuro-muscular junctional tissue, and I believe that such proof would be difficult to obtain.[1] But if we may argue from analogy, we may be justified in supposing it probable that since it resembles the other tissues in its excitatory properties, it also resembles them in the character of its response, and furthermore, that the duration of its response and refractory phase may be approximately three times as brief as those of motor nerve. This must be recognized as conjecture, but it may prove to have an important bearing on the physiology of the reflex arc, as will be indicated later.

It is a familiar fact in physiology that whereas both muscle fibers and neuro-muscular junctions are demonstrably subject to fatigue, nerve fibers respond to repeated stimulation for a long time without suffering in this respect (43). On the other hand, it is known that in absence of oxygen a nerve will cease to function sooner if stimulated than if at rest (11, p. 100). It is possible, therefore, that the difference is one rather of degree than of kind. In emphasizing the common character of the functional response in all these tissues, there is no intent to belittle the importance of contraction in muscle, an attribute of its response which is absent in nerve. There is no doubt that a wholly new functional capacity is added here, and that it constitutes the muscle's chief function. But this does not alter the fact that in the evolution of function in these tissues there is a fundamental propensity common to all.

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[1] Bazett's observations (41) appeared to indicate a very brief refractory phase in the junction, but, as he suggested, they may be open to another interpretation.

An important bearing of all this on the physiology of the nervous system as a whole is that in peripheral nerves, at least, nervous energy should not be pictured as flowing in steady streams, but in nerve impulses, each of which is a distinct event. The impulse is transient; it passes on, the tissue is then refractory, and for the next impulse must be excited anew. This conception is established for peripheral nerves; it is most unlikely that it should be otherwise with the medullated fibers in the great tracts within the brain and cord, for morphologically they differ little from the peripheral fibers. Should it be found to apply to all parts of the central conducting path the influence of this conception upon our views of the working of the brain and spinal cord would be profound. Perhaps then, the most significant course of inquiry before us is this: does the type of response portrayed by investigations on the nerve trunk constitute the basis of activity throughout the nervous system? Does every neural disturbance sweep over the conducting paths open to it, leaving them refractory, so that sustained activity must be intermittent? Or is there something at the synapse which can pass into a sustained state of activity uninterrupted by refractory phase, and capable perhaps of gradation?

APPLICATION TO REFLEX PHENOMENA. We may now consider the salient differences between conduction in the reflex arc and in the nerve trunk enumerated above, in the light of the modern concept of the functional response in nerve, and endeavor to see how far we may go, in the present state of our knowledge, along the way indicated by Lucas; that is, the interpretation of reflex phenomena in terms of the principles of conduction as we know it in peripheral tissues.

Conduction time. The first point to consider is the slower speed of conduction in the reflex arc, as judged by latency of response. The experiments of Jolly (44) and those of Forbes and Gregg (45) agree in indicating that in the flexion reflex evoked by a single shock, the time consumed within the cord is about 4 σ. In the case of the knee jerk Jolly found the time to be shorter than this, but in every reflex the rate of conduction, judging from the total distance traversed within the cord, is much slower than in the peripheral nerve trunk. This delay has its counterpart in the junctional delay in the nerve-muscle preparation. That delay occurs here is a well-known fact (11, p. 66), and if we regard the synapse as a similar junctional tissue, delay in the reflex arc implies no functional property not present in the peripheral conducting path, or at all incompatible with the fundamental principles of this type of response in general. The very much greater delay in the reflex response to weak stimuli, described by Sherrington (2, p. 21), amounting, in the case of the scratch-reflex, to 2 to 3 seconds, involves a different principle, for in these cases the response was only evoked by repeated stimulation. The problem thus becomes one of the summation of propagated disturbances and will be dealt with as such presently.

After-discharge. The second point to consider is after-discharge. Sherrington (2, p. 77) has shown in the crossed extension reflex an after-discharge, following the cessation of stimuli, amounting to more than 12 seconds. This is probably in part due to the secondary reflex effect originating in the proprioceptive impulses coming from the extensor muscle itself, but its presence even in the "de-afferented" preparation (46, fig. 16) shows that the discharge of the motor neurones may continue for several seconds after all afferent impulses have ceased to enter the spinal cord. This phenomenon appears at first sight to be at variance with the principle of peripheral conduction in which the disturbance sweeps over the tissue leaving it refractory. There appears to be instead a condition of sustained activity. But when we consider that in the gray matter we are not dealing with isolated, unbranched paths, as in the nerve trunk, but with a complex system in which one afferent fiber is probably connected with many central neurones through extensive branching (1, section II) we see that this sustained activity need not involve any fundamentally different conception of function from that developed in the case of the peripheral tissues. We need only assume sufficiently elaborate and extensive paths including chains of neurones, each adding a considerable measure of synaptic delay, to account for any observed after-discharge without introducing a functional capacity in any way at variance with the all-or-nothing principle. The apparent indications of sustained activity, in which refractory phase plays no part, may thus be due merely to the complexity of the branching paths involved.

Summation. The third difference between reflex and nerve-trunk conduction is summation. Superficially the reflex response to a series of stimuli when none occurs to a single stimulus, appears to be essentially the same phenomenon as the familiar summation of inadequate stimuli in the fresh nerve-muscle preparation. That the two phenomena are fundamentally different was first shown by Adrian and Lucas in 1912 (18), and the distinction is clearly set forth in Lucas' monograph (11, p. 54). The point to bear in mind in this discussion is that whereas in the summation of inadequate stimuli the first stimulus produces only a local effect, no nerve impulse being set up, in reflex summation we are dealing with stimuli which are adequate as far as the afferent nerve is concerned;—each suffices to set up a propagated disturbance (nerve impulse), but the single impulse fails to evoke a reflex response;—a series of impulses is required. This form of summation is therefore designated "summation of propagated disturbances."

Adrian and Lucas (18, p. 72) were able to produce this type of summation in a fatigued nerve-muscle preparation, and to determine the conditions of the conducting path on which it depended. It only occurs when the nerve-muscle junction constitutes a region of decrement sufficient to block a single impulse. It further depends on the timing of the interval between impulses, so that the second shall occur during the "supernormal phase" of recovery following the relative refractory period. Adrian (28) has since shown that this supernormal phase only occurs when the tissue is in an acid medium, but this is a state which normally results in muscular tissue from activity, and therefore presumably accompanies fatigue at the neuro-muscular junction. Adrian and Lucas (18, p. 107) were able to produce in a motor nerve with alcohol vapor an artificial region of decrement, such that a single impulse failed to pass through it, but a second impulse coming in the supernormal phase of recovery, succeeded. Summation of propagated disturbances occurs, then, when there is a region of decrement sufficient to extinguish a single impulse and when a second impulse follows during the supernormal phase of recovery, this condition depending on acidity of the medium surrounding the tissue.

Adrian and Lucas (18, p. 120) pointed out that summation in the reflex arc is clearly a summation of propagated disturbances, and suggested that it might be explained on the same basis as the summation which they analyzed in the fatigued nerve-muscle preparation, the synapse taking the place of the neuro-muscular junction. They state that although the second impulse "may fail to find its way completely through the region of decrement it will have progressed further than the first and so have opened a longer stretch of the conducting tissue to the possibility of supernormal recovery; consequently the third disturbance will in its turn outrun the second" (18, p. 119). In this way they explain the fact that in some reflex arcs a series of more than two stimuli is required to evoke a response.

Adrian (28, p. 29) has further drawn attention to the fatigability of the reflex arc and its dependence on oxygen as indications that acidity may be a common, if not a constant condition in the neighborhood of the synapse, and that such acidity as occurs there in normal life may suffice to account for both regions of decrement and the supernormal phase of recovery, the two conditions (besides the timing of impulses) requisite for summation.

From this discussion we may conclude that as far as we can see at present, not only does summation as observed in the reflex arc call for no functional propensity differing essentially from those of the nerve-muscle preparation, but that the conditions of variation in the neuromuscular mechanism which enable it to manifest summation of propagated disturbances are such as are altogether likely to exist in the central mechanism as well.

Irreversibility. The fourth point to consider is the valve-like action of the synapse whereby impulses can pass from afferent to internuncial or motor neurones, but not in the reverse direction. Sherrington (2, p. 17) suggested that this property might be the result of discontinuity of the protoplasmic path; that the "synaptic membrane," supposed to be interposed between neurones, might be permeable only in one direction to certain ions.

Another suggestion is that of Lillie (32, p. 424), based on his contention that the action current is in itself the essential cause of propagation of the nerve impulse. He suggests that irreversibility depends on the relation between the duration of the action current at one point in the conducting path and the chronaxie of the next point, which must be excited by that action current in order that the disturbance may go on. Let us examine the possibility of applying this idea to the synapse. We may assume, for example, that the time relations of excitation and response in the dendrites which conduct impulses from the synapse to the axon of the next neurone, are much briefer than those of the end branches through which the impulses approach the synapse. In order that conduction should be irreversible, it is only necessary that the action current in the end branch should last long enough to excite the dendrite, but that the action current of the dendrite should be too brief to excite the branches of the afferent neurone, because of their longer chronaxie.[3] This is, of course, speculative, but it is not at all beyond the realms of possibility, and no new functional propensity would be involved in such a mechanism. It should be noted, incidentally, that this conception does not require any transverse synaptic membrane.

Fatigue. The next point is the contrast between the great susceptibility of the reflex arc to fatigue (2, p. 218) and the indefatigability of the nerve trunk. A nerve trunk stimulated 40 or 50 times a second will continue to conduct impulses at this frequency for hours (43), (47). In contrast with this, fatigue in the reflex arc may be readily demonstrated (48).

In the spinal cat with proper controls for electrode polarization the flexion reflex may show marked fatigue in a half-minute or so. That the fatigue is central is proved by the fact that immediately after failure of reflex response stimulation of the motor nerve will evoke a full-sized contraction of the muscle.

Sherrington (2, p. 218) and Lee and Everingham (49) have shown that such reflex fatigue does not involve the motor neurone as a whole, but only the particular channel of approach to it which has been employed. Under Sherrington's direction I found (48) that fatigue of the flexion reflex as evoked through the popliteal nerve usually did not impair the response evoked immediately afterwards through the peroneal nerve, and vice versa. Indeed, the test reflex evoked through one nerve was usually even more vigorous after fatigue of the reflex arc through the other nerve than before, although when the fatiguing stimuli were above a certain strength the subsequent test reflex showed slight impairment. Sherrington concluded from evidence of this sort that the seat of fatigue is in the particular synapse whereby the motor neurone is excited (2, p. 218).

It is well known that in the nerve-muscle preparation the neuro-muscular junction is more subject to fatigue than the muscle fibers. When fatigue is established at this point there is, as we have already seen in connection with the phenomena of summation, conduction with a decrement. Reasons were mentioned in connection with summation for supposing that in this fatigued condition there is a considerable degree of acidity developed in the tissue. Thus, in the case of the nerve-muscle preparation, as well as in the reflex arc, we have reason to suppose that fatigue occurs chiefly in the junctional tissue. In the neuro-muscular junction this fatigue is associated with decrement. Both in connection with summation and, as we shall presently see, in connection with inhibition it has been plausibly argued that an important property of the synapse is conduction with a decrement. If this be so it emphasizes one more point of similarity between the reflex arc and the nerve-muscle preparation (cf. 50), and it will be altogether reasonable to suppose that fatigue acts by increasing the decrement in the synapse as it does in the neuro-muscular junction. Closely associated with the degree of fatigue and the resulting degree of decrement in the synapse, we may expect to find the degree of acidity an important factor in the operation of the reflex arc under various conditions of stress and fatigue.

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[3] Marui's observation (4) that neuro-fibrils may enter a cell body elsewhere than through the dendrites might call for a revision of this scheme, but would not prevent its development in another form.

In this connection, Stiles (51) has drawn attention to the suggestive fact that the finer branches of the neurones in the synaptic region exhibit great attenuation of the conducting substance, and that therefore we should expect any material required for the transmission of the impulse to be more rapidly exhausted here than in the larger fibers. He further remarks, "On the other hand, it should be speedily repaired because it has most extensive surface relations with the surrounding fluids."

The influence of the strength of afferent stimulation in determining its effect on subsequent reflexes, referred to above, presents a puzzling problem. The observations were as follows:

A decerebrate cat was so arranged that an extensor muscle could be reflexly inhibited through either popliteal or peroneal nerve andexcited through the opposite sciatic nerve. In most cases inhibition by a series of stimuli lasting 45 seconds applied to the popliteal nerve was found to augment both crossed extension and its reflex inhibition through the peroneal nerve if the prolonged stimulation was below a certain critical strength, but if above this strength, the prolonged stimulation was followed by depression of both excitatory and inhibitory reflexes. The graphic record of such an experiment is shown in figure 1 (48, fig. 14). It may be significant that the critical strength of the stimuli at which this change occurred in the effect on subsequent activity was in two out of three of the animals approximately the same as has since been found just sufficient to excite all the fibers in such a nerve trunk, as judged by the magnitude of the action currents. It is conceivable that for some reason which at present is not clear, prolonged excitation of all the afferent fibers in a nerve produces a generalized synaptic fatigue influencing all the synapses in the center whereby both excitatory and inhibitory effects are evoked, including those only approached through another allied nerve trunk, whereas if a considerable number of afferent fibers are not stimulated the fatigue is limited to synapses involved by those fibers that are stimulated. On the other hand, it should be noted that the approximation between the strength of stimulus which is maximal for the nerve, and that which is critical as to the central after-effect, is a very rough one at best and may be a mere coincidence. It may be that the whole effect is one of acidity; that a very slight degree of acidity in some way increases the efficiency of conduction, but greater acidity increases the decrement. A few impulses traversing the synapses might produce a fatiguing degree of acidity only in their immediate vicinity, thus limiting fatigue to themselves, and facilitating conduction in other adjacent synapses. Activity in a much larger number of synapses, especially if prolonged, might develop enough acid to cause a generalized fatigue of all the synapses in the vicinity. The recent observations of Marui (52) on central fatigue show somewhat generalized changes suggestive of catabolism, and in no way incompatible with the conception suggested above.

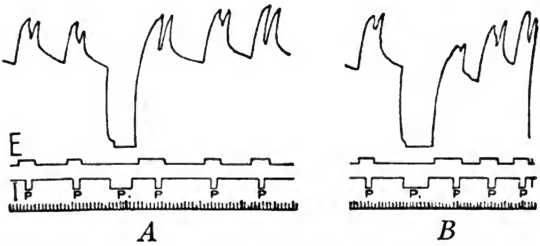

Fig. 1. Vasto-crureus muscle. Ascent of myograph line shows contraction. Rise in upper signal line shows excitatory stimulus (crossed sciatic nerve). Fall in lower signal line shows inhibitory stimulus, marked P when applied to peroneal nerve, P_1 when applied to popliteal nerve. Time below in seconds. Popliteal stimulation lasted 45 seconds in each case, drum was stopped to save space while stimulation was in progress. In A, break shocks to popliteal, 41 Z units; in B, 46 Z units.

Variability of threshold. The next point to consider is the greater variability of threshold in the case of the reflex arc than that found in the isolated nerve trunk. The assumptions as to the degree of variability of threshold in the case of a nerve trunk are generally based on the threshold of the motor nerve as judged by contraction in the innervated muscle. The motor nerve threshold thus determined appears to be far less subject to variation than the so-called threshold of the reflex arc, when stimuli are applied to an afferent nerve. This brings us to one of those points in the traditional physiology of the central nervous system most in need of a searching revision in terms of modern knowledge, and at the same time one of those points in which it is most difficult to see just how the modern conception of the nerve impulse can be reconciled with the empirical facts concerning the central structures. Histologically the afferent nerve fibers do not differ appreciably from motor nerve fibers. Furthermore, it has recently been shown (30) that most if not all of the fibers of a pure sensory nerve in a mammal obey the all-or-nothing law just as do the fibers of the frog's motor nerve. If the stimulus is applied to an afferent nerve trunk how can the threshold of the reflex be other than the threshold of the nerve? If by reflex threshold we mean the threshold of the afferent nerve, why should this show any essential difference, such as greater variability, from the threshold of the motor nerve? It is an empirical fact that the reflex threshold does show this greater variability, and it is difficult at first sight to see how the difference may be explained. The problem is presented most clearly by the experiments of Lutz (53). He showed that in the frog, with electrodes applied to an afferent nerve, the average threshold for the flexion reflex was twice as high as for the muscular response with electrodes stimilarly applied to the motor nerve. He further found (54) that when the animal was cooled the threshold for the reflex rose nine times as much per degree centigrade as did the threshold of the nerve-muscle preparation. Since he used single shocks of threshold strength, his experiment did not introduce the same opportunity for confusion through summation of propagated disturbances, which enters when repeated stimuli are used. An important conclusion is therefore indicated by his results. If each afferent fiber were connected with a single motor neurone, the pair thus constituting a single isolated reflex arc, it should make no difference in the motor response of that arc how strong a stimulus was used provided it sufficed to excite the afferent fiber. The threshold for the reflex would be simply the threshold for the afferent fiber. In a previous communication (55, p. 285) it was shown that to account for the great variability of reflex threshold compared with that of the nerve trunk, on any basis assuming isolated reflex paths, would involve a series of coincidences too improbable to be worth considering. Moreover the branched arrangement of the afferent fibers in the gray matter suggests that each one may be connected with many motor neurones, and vice versa. It is therefore misleading to picture a single afferent neurone connected with a single motor neurone as typifying the structure of the reflex arc; we should instead consider the nerve center as a place of convergence and intermingling of many separate paths (1, p. 93). This view is supported by the physiological evidence cited above in connection with synaptic fatigue. The inference is that the convergence of impulses in many afferent fibers in some way produced a different effect in the motor neurones from that produced by a few. The reflex threshold would then depend on the number of afferent fibers excited, and would no longer be identical with the threshold of the most excitable fiber among them. In this way we could readily account for great variations in reflex threshold through agencies which cause little change in the threshold of the afferent fibers.

How can this inferred influence of the number of converging impulses in the nerve center be made to harmonize with our conception of the nerve impulse? It appears to suggest a gradation of central activity incompatible with the all-or-nothing principle. Are we to suppose that at the synapse there is a different sort of activity from the nerve impulse, graded in intensity according to the number of afferent impulses producing it, its ability to excite the motor neurone depending on the intensity to which it is raised? Or are we to look on the synapse as a portion of the conducting path with the same fundamental properties as the nerve fiber, but with different time relations, and perhaps normally conducting with a decrement? Decremental conduction in itself provides the possibility of gradation of intensity in the individual impulse, as has already been mentioned in an earlier section. Yet even without this sort of gradation we may find a basis for explaining the effect of convergence of impulses at a common point in terms of the time relations of the common portion of the path. Forbes and Gregg (56, p. 221) found reasons which will be discussed presently for supposing that some part of the central conducting path has a briefer refractory period than the afferent fibers. A portion of the synaptic region, with a very brief refractory phase, common to all the afferent paths involved, would account for the effect on the basis of summation as outlined above; for even the synchronous volley of impulses set up in the afferent nerve by a single shock might arrive at the common path at slightly different times, and it would then only be necessary that the refractory phase should be brief enough for some impulses to arrive in the supernormal phase of recovery in order that they might in combination break through a synaptic resistance where without such combination they would be extinguished.

We may conclude that the great variability of the apparent threshold of stimulation in evoking reflex response points strongly to the central intermingling or convergence of conducting paths (evidenced histologically in the branching of fibers) as an essential feature in the interpretation of experimental results. We may further conclude that though these experiments offer difficulties in the way of picturing central activity in terms of the properties of functional response known in peripheral tissues, these difficulties may not be insuperable, possible ways of surmounting them being offered both by the character of decremental conduction and by great brevity of refractory phase somewhere in the central part of the path.

Mutual relations between allied and antagonistic reflex arcs. Sherrington (2, p. 175) has pointed out several instances of reinforcement of the activity of one reflex arc by stimulation of an allied arc. For example, flexion of a hind limb is evoked in the spinal mammal either by afferent stimulation of the limb itself or by similar stimulation of the forelimb on the opposite side; combined stimulation of both limbs evokes the response more easily than either stimulus alone. There are several other instances of this sort of reinforcement sometimes referred to as "Bahnung." This phenomenon appears to fall readily into the category of effects just discussed under variation of reflex threshold. If those effects can be explained by convergence of individual afferent paths, so can reinforcement. In this connection may be mentioned Camis' observation (57) that the contraction in the flexion reflex was greater if evoked by simultaneous stimulation of both popliteal and peroneal nerves than could be evoked by stimulating either one alone. This might be ascribed to the connection of the afferent fibers of the two nerves with different motor neurones; but against this view are the facts mentioned in connection with reflex fatigue. The modification (increase or decrease according to strength of stimulus) of subsequent reflex response to stimulation of one nerve following prolonged stimulation of an allied nerve suggests that both sets of afferent paths are connected centrally with the same motor neurones.

We may conclude that central reinforcement is merely additional evidence of convergence of conducting paths from various sources at common points in the nervous system.

A different relation between reflex arcs which is the exact opposite to reinforcement in effect, is reflex inhibition. This is one of the most striking and puzzling of all the phenomena in the physiology of the nervous system. It plays an important part in the "reciprocal innervation of antagonistic muscles" of which Sherrington has made such an extensive study (2, p. 83), (58), (59), (82), (60), (61), (62), (63). The elementary facts of reciprocal innervation in the limb reflexes are as follows: The normal dominant reflex responses to stimulation of an afferent nerve in a hind limb are the flexion reflex and the crossed extension reflex; the flexion reflex consists in reflex excitation of the flexor muscles and inhibition of the extensor muscles in the same limb as the stimulated nerve; the crossed extension reflex consists in reflex excitation of the extensors and inhibition of the flexors in the opposite hind limb. Under certain conditions some of these responses may be replaced by their exact opposites; this may be termed reversal. Several interesting types of reversal have been described; these will be considered later.

Reflex inhibition in its most striking form may best be exhibited in a decerebrate mammal in which all muscles acting on the knee joint except the extensor (vasto crureus) are paralyzed by section of their motor nerves. The animal is placed on its back with the femur clamped in vertical position so that the weight of the foot tends to flex the knee. Contraction of the extensor is then shown by the rise of the foot into an extended position, relaxation by its fall. The decerebrate animal will usually exhibit the sustained "tonic" contraction of the extensor muscles known as decerebrate rigidity; thus the knee will be held in an extended position. Failing this, the extensor can be reflexly excited by stimulation of the opposite hind leg ("crossed extension reflex"). In either case, if, while the knee is extended, a strong stimulus is applied to an afferent nerve in the leg under observation, the extensor muscle relaxes, allowing the foot to fall abruptly. This relaxation is part of the flexion reflex which, as Sherrington has pointed out (2, p. 229), is the obvious, defensive reaction whereby an animal in normal life withdraws its foot from a thorn or other object which causes injury. It was once supposed that this inhibition might be effected by nerve impulses going to the muscle by way of special inhibitory fibers in the motor nerve. It has long been established (2, p. 100), (64) that this is not the case; that the seat of inhibition is central, and that the condition for relaxation of the muscle is the cessation of impulses in its motor nerve.

How are we to interpret this striking phenomenon of reflex inhibition in terms of the fundamental properties of the nerve impulse? We stimulate an afferent nerve and thereby cause impulses to travel to the spinal cord, but by some strange process in the nerve center this activity is transformed into absence of activity in the motor neurones subject to inhibition.

In 1885 Wedensky (65) described a phenomenon in the nerve-muscle preparation, bearing at least a superficial resemblance to reflex inhibition, and in 1911 Lucas, in a brilliant series of experiments, established the true nature of the Wedensky effect (66). Lucas further indicated a possible way in which the principle involved might conceivably apply to reflex inhibition. Much has been written about these points in the literature (26, p. 11), (12), (55), and they are so important that it is worth while to review them here.

The Wedensky effect in the nerve-muscle preparation is as follows: If the preparation is moderately fatigued by stimulation through the motor nerve a stage is reached in which a series of stimuli of proper strength and frequency applied to the nerve evokes only an initial twitch in the muscle followed by its complete relaxation. While these stimuli are being applied, additional stimulation of the nerve nearer the muscle fails to make it contract. If the frequency of the stimuli is decreased to a certain point, it will render them effective in establishing tetanic contraction.

Lucas showed (66), (cf. also (26)) that the explanation of the phenomenon was simply this: The relaxation or "inhibition" only occurs when the neuro-muscular junction is in such a condition (fatigue) that it conducts with a decrement. The frequency of stimulation must be such that each stimulus after the first excites the nerve during its relative refractory phase following the preceding impulse, and therefore evokes a subnormal response. The first impulse of the series, being full-sized, is able to pass through the junctional tissue in spite of the decrement, and therefore evokes the initial twitch observed in the muscle, but the subnormal impulses following the first are all extinguished. Clearly, if the frequency of the impulses is reduced till the nerve is allowed to recover fully from its refractory phase each time, these impulses will no longer be subnormal: they will all be able to pass through the fatigued junction, and tetanic contraction of the muscle will result. This explains why there is a critical frequency of stimulation above which the Wedensky "inhibition" occurs, and below which it is replaced by contraction.

Wedensky suggested that this effect which he described might embody the principle involved in reflex inhibition. Lucas pointed out

(11, p. 93) that we have good reasons for supposing regions of decrement to exist normally in the junctional areas of the central nervous system, and that the only other condition necessary to establish central inhibition on the same basis as in the fatigued nerve-muscle preparation, is that the fibers leading to the region of decrement should transmit impulses with such frequency that each is subnormal, and therefore unable to pass. "A neurone so occupied would certainly be a complete block in the path of any impulses which might attempt to traverse it" (66, p. 88).

Many complications arise when we try to make this conception of reflex inhibition fit all the facts which have come to light in the study of the spinal reflexes (55). We shall consider these complications presently, but for the moment we may conclude the question of inhibition with the observation that the nerve-muscle preparation has functional properties which apparently provide a conceivable basis for explaining reflex inhibition, and that this phenomenon therefore does not necessarily imply any wholly new propensity not existing in the peripheral excitable tissues.

Under the same general heading as reinforcement and inhibition, Sherrington (2, p. 14) mentioned refractory phase and "shock" as occurring in reflex arcs in degrees unknown for nerve trunks. His argument for refractory phase in the reflex arc was based chiefly on the scratch reflex, a characteristically rhythmic series of contractions with a frequency of four or five a second in the cat, evoked by stimulation of of the skin (2, p. 45). He showed that an addition to the initial stimulus which started the reflex failed to interrupt the established rhythm, no matter when it was applied. That is, when the flexor muscle was in its relaxation phase no skin stimulus could make it contract until the contraction time arrived according to the established rhythm. More recently Sherrington and Sowton (67) have examined the refractory phase of the flexion reflex and found that it amounts apparently to only 0.7 σ. Their method was to send two stimuli into the afferent nerve at various time intervals and find the least interval at which the second stimulus was able to augment the contraction resulting from the first. Clearly a second stimulus falling in the refractory phase of the afferent nerve would fail to set up any second impulse, and would fail to augment the reflex response. It is therefore impossible by this method to measure a reflex refractory phase briefer than that of the afferent fibers. In some recent experiments I have measured the least interval between two stimuli at which the second would evoke a separate response in a mammalian nerve slightly below normal body temperature, using the action current as evidence, and found it to be approximately 0.7 σ. For this reason it is probable that in the experiments of Sherrington and Sowton the limit to the interval was set by the refractory phase of the afferent nerve, and that the refractory phase of the reflex arc was certainly no longer than that of the nerve, and possibly shorter. I have mentioned in connection with reflex threshold that reasons exist for supposing some part of the reflex arc to have a briefer refractory phase than the peripheral nerve fiber. The observations of Sherrington and Sowton are altogether in accord with this view.

The long refractory phase for which Sherrington argues in the case of the scratch reflex, introduces a somewhat different problem. In the first place it involves a wholly different conducting path from the flexion reflex, probably far more complex, and including more internuncial neurones. But more important is the fact that the scratch reflex consists of alternate flexion and extension, and therefore introduces the important factor of inhibition playing its part in the reciprocal relation between the opposed muscle groups. The failure of the flexor muscle to respond to additional skin stimuli during its relaxation phase probably depends on the reflex inhibition of its motor neurones at the moment rather than on a true refractory phase of any part of the conducting path.

The question of spinal shock is one upon which there is great disagreement among different authors. Originally Sherrington stated (2, p. 241) that spinal transection was followed by depression of all reflexes in the regions posterior to the transection, including flexor and extensor reflexes, but that the flexion reflex suffered much less and recovered more quickly than the extensor reflexes (2, p. 248). More recently Sherrington and Sowton have reported (67) that the flexion reflex in response to single shocks is actually increased by spinal transection, its threshold being lower and the contraction with a given stimulus being greater. In some experiments soon to be published, we have found the increase in the flexion reflex produced by single shocks to occur immediately after spinal transection. Sherrington originally mentioned the after-discharge as a feature of the flexion reflex which was especially impaired after spinal transection (2, p. 245). I have seen the flexion reflex in a decerebrate cat changed by low spinal transection from a small contraction with notable after-discharge to a brisk contraction with apparently no more after-discharge than appears in the twitch of an isolated muscle. Before transection, repeated stimuli caused cumulative contraction in a way that they failed to do after transection. It is quite possible, therefore, that the earlier statement about the flexion reflex taking part in the general depression known as spinal shock was due to the fact that it was then customary to use repeated stimuli to evoke all reflexes, and that therefore the cumulative effect resulting from after-discharge before transection led to a larger total contraction with this kind of stimulation than was found when the cumulative effect was abolished by transection. There is no doubt that in this reflex in the mammal the response to single stimuli is increased by spinal transection. The so-called shock effect is in this case not a depression but a modification of the reflex response. It should be noted that in the case of the frog the familiar classroom experiment for demonstrating spinal shock consists in repeated stimuli applied to the skin, which is a very different matter from single shocks applied to a large nerve trunk.

In the case of extensor reflexes there is no doubt that they are depressed by spinal transection. Sherrington has shown (2, p. 243) that this cannot be explained as a lasting inhibition due to irritation by trauma. He argues that it is due to the interruption of certain paths. Pike (68) has urged the view that normally the reflexes subject to shock involve conduction to the brain and back, and that the depression of the reflex is due to the interruption of this path; and further, that the recovery from shock depends on the gradual resumption of function by primitive spinal paths which in the course of phylogenetic development have been superseded by those involving the brain.

This is a difficult matter to settle. But we may note that the muscles whose reflexes are depressed in "shock" are those involved in decerebrate rigidity, a condition which Sherrington has shown to depend on impulses coming from the hind-brain. We may suppose that there is normally within the cord a connection between the afferent fibers from one hind limb and the extensor motor neurones of the opposite hind limb. The ease with which the crossed extension reflex can be evoked in the decerebrate animal as compared with the condition after spinal transection may depend simply on the same principle of convergence of paths which we found necessary to invoke to explain the variations of reflex threshold. On this view the recovery of this reflex from the depression of spinal "shock" would depend on some sort of "canalization" whereby the local connections between afferent and motor neurones in the cord become better able to conduct, and thus less in need of reinforcement through the converging path of descending neurones from the hind-brain. In short, we might modify Pike's view to the extent of assuming in the intact nervous system a functioning connection between the local afferent and motor neurones, but one with a decrement so great that the summation effect of impulses from the brain is required to overcome it, and further assuming in the gradual change known as recovery from shock, a decrease in the degree of decrement, enabling the same spinal connection to conduct even without this reinforcement.

Dependence on blood supply and susceptibility to anesthetics. Reflex centers are strikingly dependent on blood supply as compared with peripheral nerves, or even muscles. Interruption of the blood supply to any part of the central nervous system in the mammal results in rapid loss of function. Inadequate respiration may cause profound derangement in the spinal centers. I have seen in a "pithed" cat (entire brain destroyed) to which the air supplied by artificial respiration was inadequate, abolition of the crossed extension reflex following (and probably because of) an asphyxial convulsion (46, p. 175). In contrast with this it is well known that a mammalian peripheral nerve trunk, dissected away from its scanty blood supply, will continue to respond to stimulation for a considerable time. In some experiments on the conditions of survival of mammalian nerve trunks, soon to be published, I have found an approximately normal electric response in an excised cat's nerve kept in cool Ringer's solution, three days after removal from the animal. On the other hand, a nerve will not survive indefinitely without its blood supply, and it soon ceases to function when placed in an oxygen-free atmosphere.

A concentration of ether in the blood sufficient to abolish all ordinary spinal reflexes does not prevent the stimulation of muscles through their motor nerves. It has been shown (69) that even if ether inhalation is pushed to the point of abolishing respiration and thus causing death, the peripheral nerves will still conduct impulses. On the other hand, ether vapor applied directly to a nerve trunk will soon abolish its ability to conduct impulses, the fibers being subjected to far greater concentration of ether than they are when it is brought from the lungs by the blood stream.

Thus it appears that both in dependence on blood supply and oxygen and in susceptibility to anesthetics, the difference between the reflex arc and the nerve trunk is only one of degree. As in the case of fatigue we may possibly find the key to the difference in the consideration Stiles has brought out, the extreme attenuation of the fibers in synaptic region and the consequent relatively large extent of surface presented to the surrounding fluids. Both Lillie (70) and Troland (17, p. 341) have mentioned evidence pointing to the view that narcotics such as ether act by changing the permeability of the membrane surrounding the fiber. This view harmonizes well with the suggestion that the extent of surface in proportion to volume is what determines the susceptibility of the synaptic region to narcotics. At all events these differences do not reveal in the center any functional propensity differing essentially from those already examined in the neuro-muscular mechanism.

Gradation of reflex effect in relation to the strength of stimulus. Besides the differences between nerve trunk and reflex conduction already enumerated, Sherrington, in the *Integrative Action of the Nervous System*, mentioned the gradation of reflex response when the strength of stimulus is varied. Since that book was written before the all-or-nothing principle had been established, and since Sherrington's recent experiments (13), (14), (15) on this subject overshadow in importance the evidence discussed in the book, I shall pass over the earlier discussion and consider the more recent contributions to the subject. Clearly the all-or-nothing principle must profoundly influence the consideration of the subject, and any discussion of the matter based on the older view of graded nerve impulses has little more than historical interest.

Several researches (15), (81) have dealt with this question in the light of the all-or-nothing principle. The results of these researches have been analyzed and summarized in a recent paper (30), (cf. also (56)), in which the following general conclusions were reached: It is a striking fact that as the strength of afferent stimulation (even in the case of single shocks) is progressively increased, the magnitude of reflex response increases in a way that superficially appears to be incompatible with the all-or-nothing principle. Even after the stimuli have become so strong that the resulting action current in the afferent nerve has reached a limiting maximal value, indicating that all the fibers in the nerve have been excited, the continued increase in the strength of stimulus may result in further increase in the reflex response. The problem arises how increasing the stimulus after all the afferent fibers have been excited can further increase the reflex response. If the size of mechanical contraction of the flexion reflex and the size of action current in the afferent nerve are both plotted against the strength of afferent stimulus, it is found that when the strength is reached at which the action current ceases to increase, the reflex response practically ceases to increase over a wide range of stimulation strengths; in short, the two curves are nearly parallel. Any further increase in reflex response is usually found to be correlated with a compounding or doubling of response in the afferent nerve and electrical evidence of tetanic response in the muscle (30). The explanation seems to be that when the exciting current (even a single break shock) is strong enough it causes a local excitatory process of such intensity that it outlasts the refractory phase and is able to set up a second impulse in each fiber (56, p. 205). The conclusion is that the only possibility of gradation in the reflex response arises either in the number of afferent fibers excited or in the number of impulses set up in each afferent fiber, this latter factor entering even in the response to a single shock if this is strong enough.

In Sherrington's experiments on the flexion reflex (15) and in one of ours (30) there was evidence that even when the stimulus is not strong enough to set up more than a single volley of impulses in the afferent nerve, it may in some cases evoke in the center a disturbance which results in a repetitive discharge in the motor neurones. Sherrington (15, p. 256) considered this suggestive of a different class of disturbance in the center from the peripheral nerve impulse, a disturbance not subject to the intermittent condition imposed by a refractory phase, but capable of being uniformly sustained. The considerations already mentioned in connection with after-discharge justify us in recognizing that this view is not the necessary consequence of the evidence. The apparent capacity for sustained activity may be due to the complexity of the conducting path. At each point in the system the disturbance may be intermittent, refractory phase following activity, and yet the individual disturbances in the various branches may so overlap in time as to render the sum total of activity continuous.

In spite of the striking extent of gradation in the reflex response, there is nothing in all the evidence which is incompatible with the all-or-nothing law as regards the peripheral nerve impulse, nor is there anything which definitely excludes the same type of disturbance as the basis of central activity.

The extension of the ideas resulting from the analysis of reflex gradation to the problem of sensation has been considered in a previous paper (56, p. 229). It was shown that neither in the auditory nor the optic nerve were there enough fibers to account for the known extent of pitch discrimination in hearing and visual acuity in sight, and the known gradations of intensity of sensation in both, on the assumption that gradation depends on the number of fibers excited. If the all-or-nothing law holds good in these nerve fibers we are forced to seek another basis for sensory gradation. A possible basis was suggested by the evidence of compound or double stimulation in the peripheral nerves of the leg. An unlimited range of sensory gradation might be based on the frequency with which the impulses follow each other in the sensory fibers. Such a gradation of frequency is what would naturally result if the sensory receptor set up and maintained in the nerve endings a greater or less local excitatory process according to the intensity of stimulation, for a sustained local excitatory process, if intense, would cause the nerve fiber to respond early in the relative refractory period after each previous response, but if weak, it would allow the nerve fiber to recover each time till its threshold had nearly reached the normal level. Thus strong stimulation would set up impulses of high frequency, weak stimulation impulses of low frequency, and between the beginning and the end of the relative refractory phase would be found the range of intervals forming the basis for sensory discrimination of the intensity of peripheral stimulation (cf. 26, p. 386).

Revision of the doctrine of graded synaptic resistance. One of the most direct applications of the principles outlined above to reflex phenomena lies in the modification of the concept of graded synaptic resistance (2, p. 155). The old idea was that some synapses presented more resistance to the passage of a nerve impulse than others and, therefore, required stronger peripheral stimulation to enable the impulses to pass through. It was further assumed that if a nerve impulse had to traverse two synapses, the resistances of these would be cumulative. The first of these assumptions is obviously in need of radical revision, since it has been definitely shown that a strong stimulus produces no larger impulses in the individual conducting paths of the peripheral nerve trunk than a weak stimulus. The second assumption, that synaptic resistances are cumulative, is also untenable unless we make the improbable assumption that the axones of the internuncial neurones within the nervous system normally conduct with a decrement through their entire course, and therefore differ profoundly from the morphologically similar axones in peripheral nerves. These points need to be emphasized, for they are still to a considerable extent ignored in the recent literature. The recovery of the nerve impulse on emergence from a region of decrement into a normal region in the conducting path was clearly shown by Adrian ten years ago (9). The bearing of this fact on the doctrine of graded synaptic resistance was explicitly stated in 1915 (56, p. 227), and yet in much more recent literature (71, p. 498) the doctrine is still assumed in its original form as stated above. As

Martin recently pointed out with admirable clearness (16, p. 408), the implication of cumulative synaptic resistance is out of harmony with the all-or-nothing principle, and in particular with the demonstrated fact of recovery of the nerve impulse on emergence from a region of decrement. For if a synapse acts as a region of decrement, it will either extinguish the nerve impulse or fail to do so. If the impulse is not extinguished, but passes into a region of normal nerve fiber beyond, it will regain its full magnitude and be as able to pass the next synapse as if it had never encountered the first one. It is, of course, conceivable though unlikely that all internuncial neurones within the central nervous system do conduct with a decrement. If this were so the resistances encountered in a chain of such neurones would be cumulative, but this essential condition for adherence to the unmodified doctrine of synaptic resistance is not mentioned in those papers in which the doctrine is assumed.

RELATIONS BETWEEN SPINAL REFLEXES: *Experimental facts.* The above survey covers the more general facts concerning reflex conduction, but there are several problems in the relations between the reflex centers controlling antagonistic muscles which call for more detailed consideration. I will first describe briefly the experimental facts, and then endeavor to examine them with respect to their compatibility with the principles suggested by Lucas as a possible basis of reflex phenomena.

Sherrington, in his early work (2, p. 117) on the reflex inhibition of extensor muscles by stimulation of afferent nerves in the same limb, was led to the conclusion that such inhibition was absolute, and could not in any way be overcome by stimulation tending to produce excitatory effects in the extensor motor neurones. Later (59) he made the observation that if the strength of the stimuli applied to the afferent nerve in the opposite leg were chosen with sufficient care a partial inhibition could be demonstrated; that is, combined stimulation produced a degree of muscular contraction intermediate between the full contraction induced by crossed stimulation alone and the total relaxation induced by stimulation of the ipselateral nerve alone. This effect he called *algebraic summation* of excitation and inhibition. When such balancing or algebraic summation of central effects is produced, the partial contraction of the muscle is usually tremulous (72).

The next point to be noted may be designated *electrical reversal.* The usual effect on the extensor center of stimulating an afferent nerve of the same leg is inhibition. Sherrington and Sowton (73) found that this was regularly produced by strong faradization, but that weak faradization or, better still, stimulation with an alternating current of 20 cycles a second from a rheonome, produced, instead of inhibition, excitation as shown by contraction in the extensor muscles. Tiedemann (74) found in the frog under strychnine a similar change of central effect from excitation to inhibition on increasing the frequency of afferent stimulation.

Another significant fact is the "*post-inhibitory rebound.*" Frequently in the decerebrate preparation exhibiting a moderate degree of tonic contraction (rigidity) in the extensor muscles their reflex inhibition for a few seconds is followed, on cessation of the stimulus, by a "rebound" contraction greater than the tonic contraction existing before the inhibition (75).

Another point is *narcosis reversal.* Sherrington and Sowton showed that under certain conditions a stimulus which normally produced reflex excitation might be made to produce reflex inhibition of the same muscle under the influence of moderate chloroform anesthesia. Similar to this is the fact recently observed (55) that under light ether anesthesia the crossed extension reflex in the decerebrate animal became so modified that during the application of the stimuli the extensor contraction was only partial, but when the stimuli ceased a marked increase in contraction at once occurred, resembling the rebound described above.

Another fact of great interest is the *postural reversal* which has been discussed by Sherrington (62, p. 299) and Magnus (76). In general this means the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied. Sherrington has found that certain stimuli will cause flexion if the limb is already passively extended, and extension if it is flexed. Magnus has found that the cat's tail when stimulated at the tip is reflexly drawn toward the median plane from whichever side it happens to be hanging; thus change of initial posture shifts the contraction to the opposite one of a pair of antagonistic muscles.

The next fact to note in this connection is the *reciprocal innervation* of antagonistic muscles. Any afferent stimulus which causes reflex excitation of flexors simultaneously causes reflex inhibition of extensors. Any afferent stimulus which causes reflex excitation of extensors causes reflex inhibition of flexors provided there be any preëxisting contraction of the flexor muscles to inhibit.

Finally, there is the intrinsic tendency of the spinal centers to exhibit *rhythmic alternation* between flexion and extension, under some circumstances, even in absence of afferent stimulation. For instance, Sherrington (77) showed that the scratch reflex proceeds with unaltered rhythm in response to steady cutaneous stimulation of a remote portion of the body when all afferent fibers from the muscles themselves have been cut, showing that the alternations are not conditioned by proprioceptive impulses from the muscles taking part in the act. Graham Brown (78) showed that similar but slower rhythmic alternation (progression rhythm) occurred under a depth of narcosis such that transection of the spinal cord in the lumbar region produced no disturbance in this response, and therefore in absence of afferent stimulation of any sort.

With this summary of facts in mind let us see to what extent they may be interpreted as manifestations of functional responses of the same sort as the peripheral nerve impulse, operating under the complex conditions of the central structures.

Balancing of antagonistic effects. In dealing with inhibition, we may consider the Wedensky effect as the most promising prototype, and indeed, so far as I am aware, the only example of a similar phenomenon occurring in peripheral tissues under conditions which have been clarified by a thorough analysis, and explained in terms of the fate of individual nerve impulses. The Wedensky effect has been described and explained in an earlier section. The point to bear in mind now is that in Lucas' proposed application of the principle to reflex inhibition, it is assumed that there is an internuncial neurone through which the impulses must pass to reach the motor neurone, and that because of the decrement at the synapse between the two, there is a critical frequency of impulses in the internuncial neurone above which the effect is inhibitory, and below which, excitatory. For convenience in the discussion I have designated this the "pre-motor" neurone (55).

The fact of "algebraic summation" of central effects, described above, has been mentioned (11, p. 98), (12, p. 45) as an objection to the proposed explanation. For if reflex inhibition is conditioned by a high frequency of impulses in the "pre-motor" neurone, this neurone would be an absolute block in the conduction path as long as the frequency was maintained. If concurrent stimulation of another afferent path leading to this neurone had any effect at all, this should be an increase in the number of impulses traversing it, and this could only increase the certainty of inhibition. The conclusion would be that inhibition is absolute; the excitatory effect should fail to appear in any way. In Lucas' monograph the mention of this difficulty is followed (11, p. 99) by the suggestion that perhaps failure of complete inhibition can only occur when a considerable number of afferent fibers remains unexcited and therefore a considerable proportion of the motor neurones remains free from the inhibitory effect.

I have examined a number of old records in which the knee extensor was tested with concurrent excitatory and inhibitory stimuli of various strengths, and found that the excitatory effect never broke through inhibition except when the inhibitory afferent stimuli were too weak to excite all the fibers in the nerve (55, p. 287). We may thus dispose of this objection as far as the extensor center is concerned, and conclude that the reflex inhibition which is a part of the flexion reflex, probably does act as a complete block in those neurones in which it is established. In the case of the crossed inhibition of flexors this objection is not so easily met, but we shall presently see that here too there is a possible way of meeting it.

Electrical reversal. The electrical reversal has been cited (11, p. 96), (12, p. 44) as supporting the proposed explanation of inhibition. Apparently the facts harmonize well with the hypothesis, for in the Wedensky "inhibition" which furnishes the model, an increase in frequency at the appropriate strength of stimulus, or an increase in strength at the appropriate frequency, would serve to convert excitation into inhibition. Further support is found in the fact that Tiedemann (74), and also Sherrington and Sowton (73, fig. 3) obtained with certain strengths and frequencies of faradization an initial twitch followed by inhibition, just as is found in Wedensky "inhibition."

But further examination reveals a difficulty. The analogy implies that each impulse set up in a given afferent fiber sets up in turn a single impulse in the "pre-motor" neurone, so that the frequency of the afferent impulses is carried through unaltered to this neurone, and there determines whether excitation or inhibition shall result.

The analogy between Wedensky "inhibition" and electrical reversal of reflex effect cannot be applied so simply as this, for Sherrington (63) has shown that a single break shock applied to an afferent nerve, in evoking the flexion reflex, suffices to inhibit the extensors. This might conceivably be due to the use of a shock strong enough to cause double stimulation of the nerve, and therefore a rhythmic series of impulses instead of a single volley. On the other hand, I have evoked reflex inhibition with single shocks which showed no signs of setting up more than single impulses in the afferent nerve when tested with a string galvanometer (55, p. 292). If a single stimulus can cause inhibition, the electrical reversal cannot be explained by the simple extension of the stimulation frequency into the pre-motor neurone. This does not mean that the proposed explanation of inhibition must be abandoned, but that the requisite frequency cannot depend directly on that of peripheral stimulation; it must be set up in some other way.

In earlier sections of the paper we have repeatedly found reasons for looking to the histologically known branching of afferent fibers as a key to many phsyiological peculiarities of reflex function. We may find in the resulting convergence of afferent paths at common synaptic points a possible mechanism for the establishment of the necessary impulse frequency in the "pre-motor" neurone. This view would probably imply a much briefer refractory period in that portion of the conducting path where convergence occurred than in a peripheral nerve fiber. But other reasons exist for supposing this to be the case, as has already been mentioned in connection with reflex threshold, and will be discussed more in detail presently. Stimuli too weak to excite all the afferent fibers in the nerve, especially currents of gradual onset from a rheonome, would be more likely than maximal induction shocks to deliver impulses at a given synapse with a slow enough frequency to produce the excitatory effect.

Sherrington and Sowton (73) were unable to produce the ipselateral extensor contraction (electrical reversal) unless there was an appreciable degree of preëxisting extensor tonus, a condition known to depend on impulses from the hind-brain. This fact seems to be another instance of the need of reinforcement for the overcoming of certain synaptic resistances, similar to that suggested to account for the variability of reflex thresholds. The arrangement of branches and synapses providing for reinforcement by convergence on the one hand, and inhibition by convergence on the other, presents a confusing problem. But such an organization may be pictured, if the branches and dendrites are properly arranged and if sufficient latitude of variation in refractory periods and decrements is allowed (fig. 3).

Sherrington and Sowton suggested that the basis of the electrical reversal might lie in two different kinds of afferent fibers mixed in the stimulated nerve (73, p. 445). This would imply a difference in chronaxie between the two kinds to account for their respective excitabilities by differently timed electrical stimuli. That the reversal occurs in the case of stimuli applied to the internal saphenous nerve which contains no proprioceptive fibers (30), and that Adrian (79) found no evidence of two types of cutaneous sensory fibers with different chronaxies, are facts which are hard to reconcile with this suggestion; yet the possibility must not be overlooked.

Rebound. The increased activity of the extensors following their inhibition, is a phenomenon of great interest. The fact that it consists in a transient excess of activity in the extensor motor neurones which before their inhibition were exhibiting "tonic" activity, early suggested the idea that the tonic stream of energy had been pent up by inhibition, and on its release burst forth with renewed vigor (2, p. 212). Aside from the improbability that tonus can be dealt with in this way, the view is untenable (46, p. 160), for Sherrington (75, p. 59) found that the amount of rebound contraction following inhibition shows no correlation with the amount of tonic activity inhibited; for instance, inhibition prolonged beyond a certain time is followed by less rebound than if the inhibition is briefer. Rebound therefore cannot depend merely on the accumulation of the suppressed tonus. It has also been shown that it cannot depend solely on the flexed position of the limb (55, p. 294). Apparently rebound depends on some central effect set up by the same afferent stimulus which during its application causes inhibition.

Sherrington and Sowton, after observing the electrical reversal, suggested (80) that rebound might be due to a twofold reflex influence exerted by the stimulus during its application, the inhibitory being the dominant one, but the excitatory persisting the longer of the two after stimulation ceased.

Let us construe this in terms of our general plan of interpretation. At the outset there is indicated the same assumption made to explain after-discharge, viz., "delay paths," central neurones providing a sufficiently extended system of connected paths to account for the long persistence of activity in the motor neurones after afferent impulses have ceased to enter the cord. In addition we must find a basis for the difference in central effect during and after afferent stimulation. For the inhibition during application of the stimuli we have already assumed the necessary condition,—high frequency of impulses arriving at the pre-motor neurone from many converging paths. To account for the change to central excitation when the stimuli cease, we have merely to assume that the impulses arriving via the "delay paths" alone are below the critical frequency (cf. 55, p. 294). Thus the more direct and densely converging paths producing inhibition and the more circuitous paths through which impulses arrive less frequently, represent the two antagonistic influences exerted in the center.

Narcosis reversal. This phenomenon fits readily into the general scheme of interpretation as is shown in Lucas' monograph (11, p. 96).

It is known that lipoid-solvent narcotics cause a nerve to conduct with a decrement and that the central portion of the nervous system is far more sensitive to their action than peripheral nerves. Therefore we may expect the decrement of any part of the central conducting path to be greatly intensified by narcotics such as chloroform. If this happened at the terminal synapse a frequency of impulses in the pre-motor neurone which normally was slow enough to be excitatory, would become inhibitory. Thus chloroform would convert excitation into inhibition.

The partial suppression by ether of the crossed extension reflex during application of the stimulus, might similarly be explained by the raising of the decremental block to the critical point in some of the synapses (but not in all), and by a sequence of events similar to that assumed to explain rebound (55, p. 297).

Sherrington has pointed out that under certain conditions synaptic fatigue may serve to convert an excitatory into an inhibitory reflex effect (61, fig. 8), and that this may be explained on the same basis as reversal by chloroform. The indications mentioned above that fatigue induces decremental conduction in junctional tissues are in harmony with this idea.

Just as narcosis reversal may be explained on the basis of increased decrement in the final synapse, so the opposite reversal by strychnine (conversion of inhibition into excitation) (82, p. 288), (83), might be explained on the basis of diminution of decrement. If the decrement in these synapses largely disappears, a frequency of impulses in the pre-motor neurone, normally inhibitory, would become excitatory.

Similar effects of chloroform and strychnine found by Bayliss (84) in the case of vasomotor reflexes are significant in this connection.

Postural reversal. Bearing in mind that in general increased convergence of impulses at a pre-motor neurone decreases the chance of excitation of the motor neurones and increases the chance of inhibition, let us assume that shortening of the extensor muscle, active or passive, sets up afferent impulses of a certain frequency, and that these set up impulses in the pre-motor neurones of the extensor center. Sherrington (85), (86) has found that a contracted state passively imposed on an extensor muscle in decerebrate rigidity, is reflexly retained (shortening reaction). This would be explained if the impulses set up in the pre-motor neurones in the manner just suggested were of excitatory frequency. Those afferent stimuli whose central effect is subject to postural reversal may in themselves deliver to the pre-motor neurones in the extensor center impulses of a frequency also below the critical value. If when such a stimulus is applied the limb is in a flexed posture and no impulses are coming to the pre-motor neurones through the afferent fibers from the extensor muscle itself, then the stimulus will cause an excitatory response, resulting in extension. But if the limb is already extended, so that impulses of an excitatory frequency are already being set up in the pre-motor neurones by the afferent impulses from the muscle, and then if the external stimulus delivers to them additional impulses, we shall have the condition for inhibition, because now the frequency of impulses in these neurones will be raised above the critical value (55, p. 302).

Porter's observation (87), (88) that when one phrenic nerve has been rendered inactive by hemisection of the spinal cord, respiratory impulses can apparently be diverted across the median plane by severing or freezing the opposite phrenic nerve, and thus caused to descend the previously inactive nerve, is at first sight difficult to interpret in terms of our present knowledge of nerve function. It suggests the diversion of a stream by damming up its outlet, a concept altogether at variance with the properties of the nerve impulse. This phenomenon may be simply another case of the postural modification of central effects. The diaphragm and the abdominal muscles operate reciprocally as antagonists. The stoppage of contraction in one half the diaphragm might cause a change in the proprioceptive impulses coming from the abdominal muscles, even if no fibers were left which could bring them from the diaphragm itself, and thus account for the phenomenon on the same principles as the postural reversal of the leg muscles.

Reciprocal innervation and intrinsic alternation of flexion and extension. These two topics had best be considered together. Strong afferent stimulation in the hind limb always results in ipselateral excitation of flexors and inhibition of extensors. This is the flexion reflex and is dominant over all other limb reflexes. Stimulation of an afferent nerve in the opposite leg, if unopposed, evokes excitation of extensors; and if there be any flexor contraction going on, other than that of a maximal flexion reflex, such afferent stimulation inhibits it to some extent (61). The flexors and extensors acting on a single joint never contract fully together. Any stimulus which succeeds in producing extension will at least partly inhibit flexion (60).

We may develop a provisional scheme of connections of the spinal neurones, without claiming for it the least degree of probability in its present form, merely for the purpose of seeing whether it is possible for any arrangement to fulfil the conditions necessary to explain the phenomena in terms of nerve impulses whose properties are such as I have described. Figure 2 (cf. 55, fig. 7), representing an extensor and a flexor muscle in one limb and the neurones involved in regulating their function, shows a typical afferent nerve fiber entering the cord in that limb, one branch going to the flexor pre-motor, and the other to the extensor pre-motor neurone. Since excitation of the flexors is the dominant reaction, we may assume that the branches approach the flexor pre-motor neurone in such a way that even if all the afferent

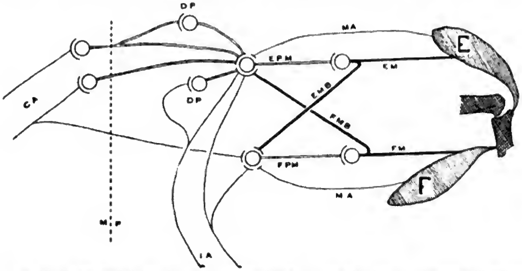

Fig. 2. Diagram of spinal neurones and their connections to provide a possible basis for analysis of reflexes. Afferent neurones shown in light lines; internuncial neurones in double lines; motor neurones in heavy lines. *E*, extensor muscle; *F*, flexor muscle; *MP*, median plane; *CA*, contralateral afferent fibers; *IA*, ipselateral afferent fibers; *DP*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *EPM*, extensor pre-motor neurone; *FPM*, flexor pre-motor neurone; *EM*, extensor motor neurone; *FM*, flexor motor neurone; *EMB*, collateral branch of extensor motor neurone; *FMB*, collateral branch of flexor motor neurone.

fibers in the nerve are excited they will not set up impulses of inhibitory frequency in the pre-motor neurone, the decrement in the terminal synapse being perhaps relatively slight. The convergence of terminal branches at the extensor pre-motor neurone, on the other hand, is such as to establish an inhibitory frequency in this neurone, provided that all or most of the afferent fibers are excited, and consequently inhibition of the extensor muscle no matter how many impulses may bearriving from other sources. Branches are shown going to other central neurones designated "delay paths" in order to account for the phenomena of after-discharge and rebound as already described. Paths are shown approaching the extensor pre-motor neurone from afferent nerves in the opposite limb. The arrangement of the branches whereby they approach should be such that the impulses set up in this neurone are below the critical value, and therefore, excitatory in effect.

To account for crossed inhibition of flexors we cannot simply invoke a convergence of impulses at the flexor pre-motor neurone in the same way that we did for inhibition of the extensors, otherwise such inhibition would establish a block which no amount of ipselateral stimulation would overcome. This would be at variance with the fact that maximal afferent stimulation produces a flexion reflex which cannot be inhibited by any contralateral stimulus. To deal with the crossed inhibition of flexors and to allow for its failure to dominate a strong flexion reflex, we may turn to structures which have been invoked by Graham Brown (78, p. 37) to explain the intrinsic alternation between flexion and extension in absence of afferent stimulation.

Von Lenhossek (89, p. 245), (cf. 1, fig. 161) has described the arrangement of the "side-fibrils" of Golgi, given off from the axons of mammalian spinal motor neurones near their point of passing from the gray into the white matter of the cord. He considered it the function of these side-fibrils to conduct impulses *toward* the axon, but his argument (89, p. 132) is based on anatomical proximity of the ends of the fibrils to the end branches of the "reflex collaterals" of the posterior roots; and this can hardly be taken as proof of "axopetal" conduction. Graham Brown suggested that this side-fibril might be concerned with the function of inhibiting the antagonistic muscle. Impulses set up in a motor neurone would traverse not only the axon but the side branch as well, and through it would reach a point where they could exert an inhibitory effect on the antagonistic motor neurones. To account for the rhythmic alternation he suggested that this inhibitory effect might become fatigued and thus in time enable the inhibited neurone to become active and in turn inhibit its antagonist. In our provisional scheme the side branch leads to the pre-motor neurone of the antagonistic center. The resulting convergence would enable impulses in the motor neurones of one center to raise the frequency of impulses in the antagonistic premotor neurone above the critical value, thus establishing inhibition (55, p. 304).

In applying this scheme to spontaneous alternation, we may simply assume that there is a steady stream of impulses from some central source (originating in the cutaneous stimulation in the case of the scratch reflex, perhaps in a "blood-stimulus" in the case of narcosis progression), and that their arrival at the pre-motor neurones is so timed as to set up in them only an excitatory frequency; whichever motor neurone responds first inhibits its antagonist in the manner just described, until a synaptic fatigue occurs at the point where the side branch acts on the pre-motor neurone; then the impulse frequency in this, falling below the critical value, reverses the process. Since contraction of one of a pair of antagonists is regularly accompanied by inhibition of the other, there is a temptation to seek a simplification of our scheme by relegating all inhibition to the side fibrils (or collateral branches) of the motor neurones. But in so doing we should fail to account for the dominance of the flexion reflex. We must recognize a difference between flexor and extensor centers. This difficulty may be met by retaining the original idea of direct convergence of afferent paths at the pre-motor neurone as a basis of inhibition in the case of the extensor center, and assuming that only in the inhibition of flexors is the activity of the side fibrils essential. The dominance of the flexion reflex would then depend on the fact that strong stimulation, exciting all afferent fibers at once, insures the necessary convergence to inhibit the extensor motor neurones and thus release the flexor center from their inhibitory action.

The question arises, how can the "electrical reversal" occur and ipselateral extension be evoked in spite of the dominance of the flexion reflex with its twofold tendency to inhibit the extensor center? Why do not the afferent impulses, even if arriving too infrequently to set up extensor inhibition by direct convergence, induce a flexion reflex and thus inhibit the extensor by virtue of the side fibrils? The answer to this question may be found in the consideration that whereas our diagram shows but one neurone of each type, actually in the spinal cord, there are thousands, and each motor neurone may send branches not to one antagonistic pre-motor neurone, but to many. The extensor center is characterized by a slower type of response, and especially by a longer after-discharge, than the flexor center (2, p. 30). In our scheme of analysis this means that its motor neurones are approached through a more extensive series of "delay paths." This arrangement may include more extensive connection for each afferent fiber, so that a small number of afferent fibers will reach the entire coördinated group of central neurones, whereas with less extensive branching in that direction a large number of afferent fibers is required to reach all the flexor motor neurones. Stimulation of an afferent nerve with the kind of weak stimuli which evoke ipselateral extension, although exciting only a few afferent fibers and through them only a few flexor motor neurones, may through the extensiveness of the delay paths set up a generalized disturbance throughout the extensor center. Then even if a few isolated flexor motor neurones have been excited, they will soon be inhibited through the side fibrils of the much larger number of extensor motor neurones in action.

In like manner we may deal with the crossed inhibition of flexor muscles. Although a flexion reflex induced by stimulation strong enough to insure excitation of all fibers in the afferent nerve apparently cannot be inhibited by any contralateral stimulation, Sherrington and Sowton have shown that a flexion reflex evoked by moderate stimulation may be so inhibited (61). We may suppose that if a sufficiently large number of afferent fibers is not excited there may be a number of extensor pre-motor neurones not subjected to the inhibitory effect. Then even if enough flexor motor neurones are excited to cause a substantial flexor contraction (as in the experiments of Sherrington and Sowton), the extensor motor neurones which are called into action will, by virtue of their side fibrils, inhibit enough of them to cause a demonstrable decrease in the contraction.

These considerations together with the synaptic fatigue invoked to account for spontaneous alternation of flexion and extension, may also account for the rhythmic alternations induced by concurrent excitation and inhibition in the extensor center (72). First one group of neurones will gain the ascendency, and then lose it through synaptic fatigue at the ends of the side fibrils.

An apparent objection to the scheme just described arises from the recent experiments of Olmsted and Warner (90). They found that when the contractions and relaxations of the antagonistic knee muscles in the decerebrate cat were registered together on a rapid drum, the latency of relaxation in one muscle was regularly longer than that of contraction in its antagonist. Extensor relaxation began 14σ after flexor contraction; flexor relaxation 19σ after extensor contraction. In the scheme outlined above we found it necessary to assume that if the extensor motor neurones were active when a stimulus was applied to an afferent nerve the flexor muscles could not contract until their motor neurones had been released from the inhibitory action of the extensor motor neurones by the inhibition of these through direct convergence of impulses at their pre-motor neurones. This view appears at first sight to be incompatible with the observations of Olmsted and Warner;

apparently inhibition of extensors should follow excitation of flexors, not precede it. This objection may be readily answered by a consideration of the contraction time of a muscle. Inhibition consists in the stoppage of impulses from traversing the motor neurones. Suppose when a flexion reflex is evoked the last impulse to traverse the extensor motor neurone and the first to traverse the flexor motor neurone occurred at the same moment, and suppose the conduction time the same in the two motor nerves. Then the latency of extensor relaxation would be longer than that of flexor contraction by the time elapsing between the initiation of the last propagated disturbance in the extensor muscle and the commencement of relaxation following it. Judging from myograph records of the simple twitch (91, p. 25), the time required for a muscle to pass the maximum of its contraction at the mammalian body temperature would be about 15σ, approximately the difference noted by Olmsted and Warner. The motor nerves are nearly the same length and their conduction times probably differ by very little; the speed of nerve reactions is so great and the distances involved in the spinal cord so small that the whole chain of processes involved in the release of the flexor motor neurones from inhibition in the manner suggested would probably occur easily within the reduced reflex time, 4σ, already noted for the flexion reflex. Therefore, the difference due to the contraction time of the muscle is so large that it completely obscures any small difference in time which may elapse between cessation of extensor motor impulses and commencement of impulses to the flexors. There is, therefore, no real objection raised by the observed difference in latencies.

It cannot be too strongly emphasized that the particular arrangement of neurones shown in the diagram is not for a moment supposed to represent the actual arrangement of neurones in the spinal cord. It is developed simply for the purpose of seeing whether any conceivable arrangement might account for the reflex phenomena without postulating new and unknown functional properties. As we shall presently see, another and quite different arrangement may be developed to answer the same purpose.

MOTOR INNERVATION IN TONUS AND OTHER SUSTAINED CONTRACTION. The frequency of nerve impulses involved in most of the activities of the nervous system on which evidence has been obtained is higher than has been generally supposed. The appearance of a characteristic action current frequency of about 50 per second in human muscles in voluntary contraction led Piper (92) to conclude that this was the frequency of impulses in the motor nerve fibers innervating these muscles. Following the lead of Buchanan (93) we have found evidence (94) indicating that the nerve-impulse frequency in voluntary innervation of the forearm flexors is much higher than that appearing in the muscular action currents. The true frequency seems to be not less than 300 per second.

In contrast with this finding is the recent observation by Gasser and Newcomer (95), confirming those of Dittler (96) and Garten (97), that in the dog the action currents in the diaphragm correspond exactly with those of the phrenic nerve simultaneously recorded, the frequency being about 100 per second. It is interesting that the evidence should indicate such a difference in the mode of innervation of two muscles, both under voluntary control. No reason for the difference is known, so far as I am aware.

The known refractory phase of medullated nerves at mammalian body temperature is such that it should set an upper limit to their nerve-impulse frequency in the neighborhood of a thousand per second. If the internuncial neurones do not differ greatly from the peripheral nerves we should expect to find the critical frequency in the assumed pre-motor neurone somewhere between 1000 and 300 a second, judging from the observed duration of the relative refractory phase in motor nerves.

The requirements of the proposed scheme suggest a briefer rather than a longer refractory phase in the pre-motor neurone than is found in peripheral nerves; therefore when reflex excitation occurs the disturbances should be available for exciting the motor neurone at least as often as it is able to respond. Therefore we should expect the frequency of impulses in the motor nerve to lie somewhere between the limits mentioned, viz., between 300 and 1000 per second. This conclusion is in harmony with that arrived at by experiment in the case of human forearm flexors in voluntary contraction.

Tonus. Tonus is a baffling phenomenon, especially when one attempts to interpret it in terms of propagated disturbances in the tissue which exhibits it. It is a well-known fact, which has often been cited (98), (99, p. 535), (100) in connection with the innervation of skeletal muscles, that in the mollusc, pecten, there are two distinct types of muscle, one capable of executing movement, the other a "postural" muscle apparently not concerned with movement, but capable of maintaining its contracted state against great force without expenditure of energy, thus dynamically resembling a vise. Sherrington (98) has noted the striking similarity of behavior between the extensor muscles in moderate degrees of decerebrate rigidity and the postural muscle of pecten. The condition he describes is one that he calls "plastic tonus." The muscle assumes any degree of shortening passively imposed on it, and maintains the posture for a long time, apparently without fatigue. Roaf (101) found no evidence of increased metabolism in muscles during decerebrate rigidity; and Bayliss (102), measuring heat production, found evidence of great economy of energy in this condition. On the strength of these data Wilson (100, p. 558) states that "the evidence of duality of muscle function in vertebrates appears satisfactorily demonstrated." (cf. Kahn (112) and Einthoven (113).

What can be the basis of such a duality of function? We know that the state of decerebrate rigidity depends on nerve impulses, both afferent and efferent; it is a reflex. Cutting either the afferent fibers from an extensor muscle or the motor fibers innervating it abolishes the "tonus" (2, p. 301). Some influence passing along the motor nerve must maintain it. The only influence we know of that can traverse a nerve is the nerve impulse or propagated disturbance whose properties I have described—a definite, transient change, giving no evidence of qualitative variations. The nerve impulse is what calls the muscle into action when the nerve is artificially stimulated; it is supposed to be the means of evoking voluntary and ordinary reflex contractions in the muscle. But "tonus" is supposed to be evoked by something different. Are we to assume that the nerve is capable of transmitting another wholly different kind of influence of a character as yet unknown? Or may we yet find a basis for explaining "tonus" as well as other reflex acts in terms of the well-recognized propagated disturbance?

Buytendyk (103) has shown that in decerebrate rigidity action currents may be led off from the extensor muscles showing practically the same frequency as those of voluntary contraction, and varying in amplitude rather than in frequency in various degrees of rigidity. This result tends to indicate that reflex "tonus" is not fundamentally different, as regards the underlying type of disturbance, from voluntary and other reflex contractions. Experiments, of which a preliminary report has appeared (104), fail to show other than a quantitative difference between the crossed extension reflex, post-inhibitory rebound and the tonic contraction of decerebrate rigidity, as regards motor innervation. They do not, however, prove definitely that qualitative differences may not exist.

There is a conceivable basis for explaining the extraordinary economy of energy in reflex tonus as compared with voluntary contraction, arising from the explanation of the "shortening reaction" suggested above.

Barbour and Stiles (105) have found evidence indicating that sustained reflex contraction sometimes consists in alternate periods of activity and rest in individual muscle groups. Possibly a group of fibers in a shortened state, either by virtue of their own contraction or of those about them, sends to a limited number of motor neurones the requisite proprioceptive impulses to establish reflex contraction. The muscle fibers thus excited may be different from the first group. Synaptic fatigue (perhaps at the junction of the afferent fibers with the premotor neurones) releases these muscle fibers before they become subject to fatigue, but meanwhile their contraction has reflexly evoked that of a third group. And so the fiber groups may take up the load in rotation and, for some reason, by this means attain an economy otherwise impossible (cf. 111).

It is obviously unwarrantable to consider the evidence at hand sufficient to establish such a mechanism of rotation as that just described; it is merely mentioned to show how many possibilities arise in a system so complex as we know the neuro-muscular mechanism to be. The object is to show that the same kind of propagated disturbance which has become familiar in isolated nerve and muscle, may be the basis of the baffling phenomena of reflex tonus, the apparently fundamental differences in the kinds of activity observed depending in reality on the vast complexity of organization in the system as a whole. The conclusion to be drawn is that in spite of the striking resemblance between plastic tonus in skeletal muscles and the postural or "catch" muscle of pecten, we are not justified. without further proof, in assuming it to depend on a function in muscle distinct from that involved in "tetanus," evoked in turn by a special function in nerve distinct from the ordinary nerve impulse.

Parker has suggested that the possible function of the neuro-fibrils has been somewhat overlooked in physiological discussion, and that conceivably these may be concerned with the transmission of such special impulses as may induce tonus. This interesting suggestion is one which is difficult to approach by experiment, but possibly it finds support in the observation of Lucas (27) that the nerve in the crayfish claw contains two "excitable substances" of widely different chronaxie.

The theory that tonus in skeletal muscle depends on innervation through the sympathetic nervous system (114) has been shown to be without adequate foundation (115), cf. (116).

A PHYSICAL VIEW OF FUNCTIONAL DIFFERENCES. Looking at the nerve impulse from the point of view of the physical properties of the fiber, we may find considerations which possibly will clarify the problems of reflex conduction. Reasons have already been given for concluding that essential features in the conducting function of the nerve fiber are the polarized state of the membrane at rest, and the breaking down of this polarization (difference of potential inside and outside) in activity, the depolarization being propagated by means of the resulting action current. Lillie (32), comparing different nerves and muscles, and Lucas (106), observing a muscle at different temperatures, found cogent reasons for concluding that the velocity of conduction depends on the speed of development of the electric response at a given point in the fiber. Lillie reinforced this conclusion with measurements showing that the action current in nerve is just about strong enough to account for the actual velocity of the impulse on this basis (32, p. 434).

Williams and Crehore (107) attempted to explain the nerve impulse as a transient electric current conducted as in a cable. Their explanation as originally formulated proved untenable for reasons which have already been mentioned (p. 367). But certain facts were brought to light in their investigation, which must have an important bearing on the problem. They found that the distributed capacity of a motor nerve fiber as estimated from its histological structure and the known chemical properties of the myelin sheath, together with the ohmic resistance estimated from measurements of a whole nerve, suffice to account for the transmission of a transient electrical current in such a conductor as the fiber appears to be, at the same velocity as is actually found in the nerve impulse. The agreement is remarkably close; furthermore the difference between the velocity of conduction in medullated and non-medullated nerves is the same sort of difference that they would expect on theoretical grounds because of the greater capacity involved in the case of the thinner sheath.

At first sight Lillie's view that velocity of conduction depends on the rate of development of the action current appears to place the cause of the velocity in something quite distinct from the physical constants, resistance and distributed capacity; the two views seem to have nothing in common. But when we consider what the necessary effect of resistance and capacity must be on such a conductor we see that we must deal with the two ideas together. The question arises, what determines the speed with which the action current develops at a given point in the fiber? Suppose the speed of conduction does depend on the rate of rise of the action current, can this electrical disturbance be conducted along such a conductor faster than the capacity and resistance would permit the crest of a transient current introduced from an outside source to travel? Both resistance and distributed capacity retard the progress of a transient change of potential imparted to an insulated conductor. The structure of the nerve fiber is such that there must be resistance and distributed capacity in it of the general order estimated by Williams and Crehore. These physical constants must act in the nerve as in a cable, retarding the development of a potential change at any given point, and therefore retarding the velocity of transmission. Thus the rate of rise of the action current and the correlated velocity of conduction must together be controlled by these physical constants. On this view one function at least of the relatively thick myelin sheath in a medullated fiber would be to insure high speed of conduction, a result of obvious utility in the rapid coördination of movement.

In emphasizing the community of functional capacity in all the excitable tissues, I called attention to the approximate agreement between quantitative variations in the duration of the different aspects of function—chronaxie, action current, and refractory phase. Turning to the structural differences in the known tissues we may look for a basis of these differences in the dimensions of the different structures which must determine in part the resistance and capacity, and we may further attempt to correlate velocity of conduction with the other quantities on the same basis. Besides these constants we must consider also the ease with which excitation occurs, i.e., the intensity of the action current which must occur at a given point in advance of the crest of the approaching wave in order to excite the tissue there. In general we find that those tissues which are rapid in one aspect of their function, tend to be rapid in others. Muscle, as compared with nerve, has a long chronaxie, long action current, long refractory phase and slow conduction. The thin sheath and consequently large capacity might well account for this. The comparison of medullated and non-medullated nerve fibers shows similar differences. On the other hand, Adrian (42) has shown a lack of parallelism between nerve and muscle in the comparison of refractory phase and action-current duration. If we knew the three determining factors,—resistance, capacity and local excitability in each case we might find in them the basis for this divergence.

From the point of view of reflex function it will be of interest to see if these concepts can throw any light on the properties of the attenuated portions of the nerve fibers in the synaptic regions. We have already seen several functional properties wherein synapses resemble the junctional tissue in the neuro-muscular mechanism. It would go far toward removing functional characters from the realm of the unknown if we could find a probable basis for them in the dimensions and resulting physical constants of the structures involved.

The nerve-muscle junction has been shown to have the briefest chronaxie of all the tissues examined. I have already mentioned Bazett's observations which pointed to a correspondingly brief refractory phase in this tissue, and the likelihood of a correspondingly brief duration of response. Its speed of conduction, however, instead of being rapid, as we might infer by analogy, is slow compared with nerve conduction, for a delay in transmission is demonstrable there. We do not know its finer structure well enough to seek an explanation of these facts in terms of the probable physical constants.

In making a comparison with the synapse we may start from the observed facts indicating many physiological resemblances between it and the neuro-muscular junction,—decrement, fatigue, delay, etc. Forbes and Gregg were led by their observations (56, p. 221) to infer that in some central part of the reflex path the refractory phase was briefer than in the peripheral afferent nerve fiber. This inference seemed necessary to account for summation of propagated disturbances on which apparently depend the observed variations of reflex response to graded afferent stimuli. This idea fits in well with the other apparent similarities between the synapse and the neuro-muscular junction. We do not know the structure of the synapse in sufficient detail to apply physical principles to conduction in it with much confidence of accuracy, but we may attempt a trial hypothesis based on such properties as are histologically apparent. One point we are sure of,—great attenuation of the finer terminal branches and of branches of the dendrites. The question of contact or protoplasmic continuity between neurones has been much debated. Sherrington (2, p. 16) has been inclined to ascribe most properties of the synapse to a synaptic membrane which may be looked on as transverse, i.e., interrupting the continuity of the conducting path. Marui (4) maintains that the neurofibrils of one neurone pass directly into the cell body of the next. It has already been noted that Lillie's explanation of irreversible conduction at the synapse does not require a transverse membrane (32). Conceivably neurones, though arising as separate cells, establish protoplasmic fusion in the development of functional reflex arcs, possibly by a process similar to the formation of foreign body giant cells (108). From the point of view of the membrane theory of nerve conduction it is easier to picture transmission of the impulse through a reflex path if we assume the membrane surrounding the conducting unit to be continuous, and not interrupted by a transverse membrane. It will be of interest to see if the properties of the synapse could depend on variations in the dimensions and the resulting time relations of different portions of the path, rather than on a transverse membrane, especially in view of the doubt cast on the existence of such a structure by Marui.

Applying the principle of physical constants to the synaptic region we may be certain that one result of attentuation will be a great increase in the ohmic resistance per millimeter. This will tend to retard conduction. The distributed capacity will depend on the ratio of internal to external diameter; a thin sheath in proportion to the total diameter of the fiber confers a large capacity. So far as I know, data are not available for estimating whether capacity would undergo increase or decrease as the fiber splits into its finer branches. Small capacity would tend to enable the electric response to develop rapidly at a given point. Therefore if the sheath continues to be as thick in proportion to the total fiber as in peripheral nerve, conditions may exist for a brief duration of response, and yet conduction would be relatively slow because of the high resistance. We do not know what determines the duration of refractory phase, but in general it shows fair correlation with duration of response (42); furthermore we should expect the exhaustion and renewal of material, such as might determine the refractory phase, to occur more rapidly in a small structure than in a larger one (51). Therefore there is some likelihood on various grounds that the fine end branches may be characterized by brief chronaxie, response and refractory phase as compared with the larger peripheral fibers. Table 1 shows the approximate dimensions and other data bearing on the possible influence of these dimensions upon function. The dimensions are taken from Lewis and Stöhr (109), the times are roughly estimated for temperatures between 15° and 20°C., from the data of Lucas (5), Lapicque and Legendre (110), (cf. 99, p. 401), Lillie (32) and Adrian (42).

The next question is,—what will happen if a nerve impulse conducted in accordance with the principles assumed, encounters a region where the fiber splits into a number of finer branches? According to the principle of Lillie which we are at present assuming, the electric disturbance in the last part of the full-sized fiber is the cause of excitation in the smaller branch; if this electric disturbance is of such long duration that it continues to be above threshold value till after the response and the ensuing refractory phase in the more rapidly acting branch, it will set up a second response in the latter; if the branch has a brief enough refractory phase it may respond thus three or four times to the action current in the main fiber. Thus a single nerve impulse entering the terminal branches may conceivably be broken up into a series of briefer impulses. Conceivably this phenomenon occurs at the neuro-muscular junction, and may provide a basis for explaining the increased delay in conduction of an early second response from nerve to muscle, described by Lucas (39). Experiments are now in progress bearing on this point. They do not go far toward revealing whether this phenomenon occurs or not, but at present the results are in harmony with the hypothesis.

Let us see if such a mechanism can form a basis for explaining the various reflex phenomena, and thus enable us to dispense with the confusing transverse membrane, and possibly also to simplify the arrangement of neurones. For example, we may assign different frequencies of response to various positions of the dendrites, and include in them the necessary regions of decrement, thus dispensing with the "pre-motor" neurone altogether.

TABLE 1

TISSUE	SKELETAL MUSCLE	MEDULLATED NERVE	NON-MEDULLATED NERVE	β-SUBSTANCE	HYPOTHETICAL SYNAPSE
Total diameter of fiber	30μ	20μ	3μ	?	?
Sheath	Thin	Thick	Thin	?	?
Resistance	Low	High	High	?	Very high
Capacity	Large	Small	Large	?	?
Chronaxie	4.0σ	0.3σ	20.0σ	0.04σ	?
Rise of action current to maximum	2.5σ	0.5σ	50.0σ	?	?
Velocity of conduction (meters per second)	1.5	30.0	0.2	Slow	Slow
Absolute refractory period	4.0σ	2.0σ	?	Brief?	Brief?

In order to illustrate how this idea may be applied, let us consider a specific problem mentioned in connection with electrical reversal and its dependence on tonus, viz., the arrangement of branches providing for reinforcement by convergence of impulses at one point and inhibition by impulses approaching from another source. Let figure 3 represent a motor neurone with the converging branches of its dendrites, each in connection with an afferent or internuncial fiber; let *A* and *B* be the paths whose impulses reinforce each other on the principle of summation; let *D* be the path through which inhibitory impulses arrive.

The decrement in C, where A and B meet is such that a single impulse fails to pass; the refractory phase there is very brief. It frequently happens that impulses arrive through A and B at the right interval to cause summation, the second falling in the supernormal phase of recovery in C. A second impulse so timed will pass the remainder of the dendrite and set up an impulse in the axon F. Any impulse approaching through the afferent fiber D, when reaching d, the most attenuated part of that path, will be broken up into a series of very brief impulses whose frequency is so high that each reaches the larger main trunk of the dendrite E early in its relative refractory phase following the preceding impulse, therefore the disturbances in E will be subnormal and fail to pass such decrement as exists in E. Thus as long as impulses approach through D, E is the seat of a total block to impulses coming from all branches of the dendrite.

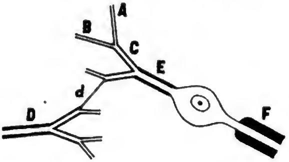

Fig. 3. Diagram of motor neurone with hypothetical arrangement of conducting paths. See text.

This principle may be applied to a general scheme for the organization of the spinal centers, shown in figure 4, which is simpler than that in figure 2, and yet I believe, provides for all the phenomena called for in the discussion. A great difficulty in the original scheme was to picture a pre-motor neurone with so brief a refractory phase as to respond with separate impulses to the rapid sequence of afferent impulses reaching it almost simultaneously. If we dispense with the assumption of convergence as the basis of inhibitory frequency in the extensor center, and refer this frequency instead to the breaking up of a single impulse into a series of briefer ones in the finer branches, we may establish the conditions for inhibition on the principle just explained in the schema shown in figure 3. The rest of the scheme will operate substantially in the manner outlined in connection with figure 2, with the modification that excitation in the extensor center depends largely on the summation effect of convergence just explained (fig. 3). The release of the flexor motor neurones from inhibition by the side fibrils of the extensors in the case of a dominant flexion reflex will depend on inhibition of the extensors. The same considerations of preponderance of numbers of neurones will apply as before, in the case of crossed inhibition of flexors.

This simplified scheme presents whatever advantage there is in unification of the principles involved. It affords a possible, if not probable, cause of the various impulse frequencies, instead of merely postulating them.

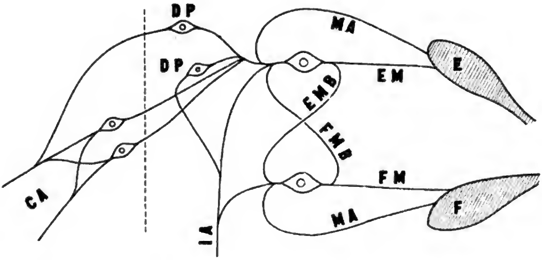

Fig. 4. Simplified scheme of spinal neurones. Lettering as in figure 2.

CONCLUSION

Any elaboration of a schema such as those presented is apt to be construed, in spite of earnest professions to the contrary, as an attempt to endow a provisional hypothesis with the dignity of a well-founded theory. Again I wish to emphasize that neither schema as outlined is advocated as being at all probable. They are developed merely as samples of the kind of possible arrangement whereby we may test the question whether our present knowledge of reflexes reveals anything that cannot be reconciled with Lucas' proposed interpretations.

Our view of so large a problem must necessarily remain inconclusive until our knowledge has advanced far beyond its present stage. But when we consider the enormous complexity of connections in the nervous system, and the great range of possible variation in the time relations of response in the different parts, depending on structure and dimensions, we may well believe that a basis for all the great diversity of function may lie in the single type of disturbance which seems to be a phenomenon common to nerve and muscle fibers. We should therefore refrain, at least for the present, from concluding that summation, inhibition, tonus, and all the other phenomena characteristic of the central structures require the assumption of new and wholly unknown functional capacities in the central mechanism.

The question raised by Lucas is still unanswered. But if the time comes when it can be shown that the evolution of the dominant activities of man has depended essentially on the elaboration of a single process, the development of the branched and attenuated fiber with an unstably polarized membrane appearing in enormously varied forms, but ever the same in its basic properties, this will be a generalization of the first magnitude.

Such a generalization need not affect any philosophical position we may take as to the relation between cerebral activity, as viewed from the objective standpoint, and consciousness as known to us through our own subjective experience. It should not seek to rob conscious life of any of its subjective properties. It would merely, if substantiated, effect a simplification of the physical aspect of the activity of the nervous system.

I wish to express my thanks to Miss M. Taylor and Mrs. G. B. Ray for help in preparing the work for publication.

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AUTOLYSIS AND ATROPHY

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Atrophic changes in mass of body tissues are brought about by chemical liquefaction of the tissue proteins, catalyzed by enzymes present in the cells. In the course of this liquefaction the same products are formed which appear as the end result of hydrolytic cleavage of protein by the enzymes of the digestive tract, namely, the peptides and amino-acids. By diffusing out into the blood and lymph, these products are removed and the cell or the tissue decreases in mass and in metabolism to a proportionate degree. In certain atrophies, digestion of the tissue proteins is further facilitated by the action of phagocytes, but even in these cases phagocytosis is a secondary process, and must be preceded by the initial steps of autolysis or post-mortem changes which produce chemotactic substances attractive to the phagocyte.

The early work in autolysis has been so ably reviewed elsewhere by Levene, Wells, Dernby, etc., (1), (2), (3), (4), (5) that in this paper we may consider it only in its broadest aspects and omit the detail. This becomes all the more necessary in view of the difference in attack and technique in the earlier work, the neglect of certain factors such as the H-ion concentration, which is of fundamental importance, and the influence which point of view seems to have exerted in the collection and interpretation of data in certain instances. Those pieces of work have been selected for discussion which have contributed important steps to the advance of our knowledge of the mechanism of autolysis and atrophy.

The earliest scientific study of the phenomenon of autolysis was that of Salkowski (6). He described the process correctly as one of self-digestion of the tissue proteins and pointed out its fundamental identity with digestion in the alimentary tract. It is doubtful however whether Salkowski and his students (7), (8), (9) appreciated the importance of this phenomenon in its relation to intermediary metabolism. It appeared as an interesting post-mortem disintegration. Salkowski named the process "auto-digestion" and showed it in liver, spleen and other tissues by the production of leucine and tyrosine, which could be isolated and identified.

In 1900 Martin Jacoby (10) published his classic papers on autolysis and its relation to the atrophic disintegration of the liver in phosphorus poisoning. Later a third paper (11) appeared in which the specific action of liver protease was shown. Jacoby approached the problem from the angle of the pathologist and was therefore struck by the outstanding facts that *a*, normal liver autolyzes, producing typical end products of protein cleavage; *b*, phosphorus-poisoned livers digest more rapidly and more completely than the normal; *c*, enzymes may be liberated into the blood stream during the acute atrophy of phosphorus poisoning, which digest fibrinogen, so that the blood may not clot at all, or if it does clot, the fibrinogen speedily liquefies again; *d*, the process goes on with the animal and tissue still alive, and must therefore be of much greater import than as a post-mortem phenomenon only.

Much of the subsequent detailed work in this field was anticipated by Jacoby. He separated the enzymes from autolyzing liver by precipitation with ammonium sulphate and showed that they accelerated the decomposition of a normal liver mixture. He observed that the residue after prolonged autolysis consisted of nucleins, connective tissue debris, and a soluble albumin. The globulin fraction had entirely disappeared. The amino-acids do not accumulate in a liver with circulation intact, but if a lobe be tied off for several hours in the surviving animal they can then be found. Even the liver of the phosphorus-poisoned dog shows no amino-acid accumulation until the agonal stage is reached, when the hepatic circulation is much depressed. Although phosphorus poisoning of the liver leads to its rapid disintegration *in situ*, the addition of phosphorus to a liver hash does not alter its rate of autolysis. There appears to be a certain specificity of action of the liver enzymes, since they do not digest lung tissue. On the other hand, if proteoses and peptones are added to a liver hash, these are digested by the liver proteases. This observation evidently anticipates the discovery of erepsin in tissues. Jacoby used antiseptics in his experiments, particularly an excess of toluol.

Following Jacoby's publications, the field of autolysis at once attracted investigators, and the next two decades saw an enormous accumulation of data concerning this mechanism.

The technique used varied with different investigators. In the main it consisted in grinding tissues fine, adding water and an antiseptic, or attempting to carry on the process under aseptic conditions. An initial sample was analyzed, usually by coagulating the proteins with heat and testing the filtrate for total nitrogen, proteose, peptone, amino-acids, ammonia, etc. Or precipitating agents were used to remove protein, such as tannic acid, colloidal ferric hydroxide, trichloracetic acid, etc. This was an improvement since the hydrolysis of the proteins during heat coagulation must always introduce some uncertainty in the interpretation of the results.

Some interesting work was done on the press-juice of tissues rather than the comminuted organ. Results obtained on such material however cannot be taken as representing the autolytic picture in the whole tissue, so that it loses some of its value. This fact is clearly seen in the results of Hedin and Rowland (12) on muscle press-juice. This material digests about as well in alkaline media as in acid, while we find that whole skeletal muscle behaves like other tissues, digesting best in acid media, and being inhibited in alkaline.

The results obtained under aseptic conditions, rather than with antiseptics present, must always remain open to the suspicion that the digests were not sterile. Even where routine cultures were found negative, it does not by any means prove that bacteria were not present, as was shown by Wolbach, Saiki and Jackson (13), (14). These authors found evidence that bacteria may be present regularly in dog liver, which require however very special culture media to grow them outside the liver tissue itself. Wherever hydrogen sulphide is noted as a product of autolysis, or where the liver mass turns dark, as described by Magnus-Levy (15), the presence of bacteria is practically certain. Thus a considerable number of papers on autolysis must be accepted with reservation on this account.

On the other hand, the antiseptic digest must be equally carefully scrutinized since antiseptics may alter the autolytic rate very materially. Boric and salicylic acids unquestionably accelerate the process (16) so that the autolysis is exaggerated far above that for the normal tissue. Apparently one of the best antiseptics thus far described is toluol, which does not alter the proteins nor destroy the enzyme, nor greatly alter the speed of the reaction in either sense, but which is sufficiently active as a germicide to inhibit bacterial growth, if the mass is kept saturated with it. Jackson (17) found that toluol decreased autolysis considerably but his control digests were admittedly infected so that the greater speed of the aseptically set up digests is to be attributed to the action of bacteria. In practice it is found that shaking a digestion bottle with toluol, present to the extent of about 10 per cent by volume, every few hours for the first twenty-four, suffices completely to destroy or inhibit bacterial growth. Such a digest will then remain for years in the thermostat without the slightest evidence of putrefactive change.

Autolysis may be measured by the amino-acids produced, or by intermediate cleavage products. The two fractions represent essentially different phases of the process. As a criterion of total autolysis, the amino-acids are undoubtedly the best. They may be titrated directly by the Sörensen method (18), or decomposed by the Van Slyke technique (19), or they may be estimated indirectly by depression of the freezing point (20) and by conductivity determinations (21). If ammonia is split off in the process, it will be titrated as amino-acid by the Sörensen method, of course, so that allowance should be made for ammonia production in strictly interpreting amino-acid titration figures. The products of primary cleavage are precipitated by tannic acid, trichloracetic, or phosphotungstic acid from the filtrate from heat coagulation, and the total nitrogen determined. Inasmuch as primary protein cleavage liberates tyrosine-containing peptides which will react with the phenol reagent of Folin and Denis (22), this method may be used as a colorimetric estimation of initial cleavage (23).

Ammonia liberated in the course of autolysis can easily be estimated by the aeration technique, Nesslerizing the distillate. Some of the early work indicated that ammonia was split off in extraordinary amounts during autolysis, and this mistake is widely quoted in the literature. Where unusual ammonia values were obtained, putrefactive processes were probably going on (24). Later work (25) has clearly shown that deaminization of the amino-acids does not take place to any extent in the *in vitro* experiments. Ammonia is produced slowly, increasing over a long period of time. It is not formed from amino-acids but from acid amides decomposable by hydrochloric acid. Dakin showed that if the total acid amide in kidney digests was determined by heating with strong hydrochloric acid, autolysis merely increased free ammonia at the expense of the acid-amide fraction. Deaminization of amino-acids probably requires the active oxidative reactions of living tissue. While the deaminization of purine bases occurs (26), (27), (28), the amount of ammonia derived from this source is not large.

Results of earlier work. The more important results of earlier work in this field, weighed critically in view of the technique employed, established the following important generalizations.

1. All tissues examined (the connective tissue group is not included) will autolyze to a greater or less extent (29). The epithelial tissues such as liver, thymus, thyroid, mammary, salivary, kidney and other gland tissues, hydrolyze most rapidly and most completely. Muscle tissue autolyzes to some extent, but much less than gland tissue. Heart muscle stands intermediate.

Vernon (30) showed that the ereptic activity of mammalian tissues had the following relations:

TISSUE	ACTIVITY (MEAN)
Kidney	14.3
Pancreas	6.4
Spleen	7.6
Liver	5.0
Cardiac muscle	1.59
Skeletal muscle	0.77
Brain	1.24
Gastric mucosa	3.9
Duodenal mucosa	27.7
Jejunal mucosa	18.2
Ileal mucosa	14.4
Large intestinal mucosa	5.8

While this table gives only the ereptic values it nevertheless shows approximately also the total comparative autolyses.

2. Tissues like the liver, kidney, thymus, etc., are found to autolyze more rapidly in the presence of quite a variety of substances. A partial list of these accelerators includes the sulphate, oxalate and chloride of iron, the chloride, sulphate, acetate and lactate of manganese, gold and platinum chlorides, aluminium sulphate and chloride, cobalt chloride and nitrate, lead acetate and nitrate (31), (32), (33), (34), (35). The following compounds of arsenic have been reported as accelerating the reaction when present in minute amounts: As_2O_3, Na_2HAsO_3, K_2HAsO_3, $CaHAsO_3$, Na_3AsO_4, K_3AsO_4, $AsCl_3$, $AsBr_3$, AsI_3 Aa_2S_3 and several others (36). In general all of the acids which do not precipitate the proteins are found to accelerate the reaction, even including such very weak acids as boric, salicylic, benzoic, carbonic acid and hydrogen sulphide (39), (40), (41), (42), (43), (44), (45). Iodine and bromine in small amounts accelerate autolysis, while chlorine has not been tried (46), (47) (48). A large number of inorganic hydrosols are reported as accelerators by Ascoli and Izar (49), (50), (51), (52), (53), (54). They include colloidal Ag, Pt, Au, Hg, Pb, Fe, Cu, Pd, Ir, $Fe(OH)_3$, MnO_2, $Al_6O_{11}H_{10}$, As_2S_3 and Sb_2S_3. Silver nitrate and lactate, and mercury salts in very small amounts, are also found to increase autolysis (37), (38).

It is clear that the majority of the substances reported are either acids or hydrolyze in water to form acids. The other groups of accelerating substances must depend on some other factor, less obvious, for their accelerating effect.

3. Tissue autolysis may be definitely prevented or inhibited by $NaHCO_3$, $NaOH$, $CaCO_3$, ZnO, MgO and other basic compounds which either make the brei alkaline or keep it neutral (55), (56), (57), (58), (59), (60), (61) Other inhibitory agents are the ions of some of the heavy metals at considerable concentrations which may combine with and precipitate the proteins and also probably the enzymes; arsenious oxide in considerable amount, and formaldehyde (62). Alcohol at fairly high concentrations inhibits, but autolysis may continue slowly in surprisingly high percentages as was shown by Wells (63).

4. Some foreign proteins introduced into a liver brei are digested (64), (65), (66), (67), (68), (69) Gelatin, proteose and peptone are digested rapidly, casein somewhat less readily. While some other foreign proteins digest, the evidence is far from complete in establishing the availability of foreign proteins in general for hydrolysis in autolyzing tissue mixtures, and no very definite conclusions are warranted on the subject of specificity. Some protein preparations definitely inhibit autolysis as for example blood serum and egg albumin (70), (71), (72), (73), (74)

In brief, the speed of the autolysis and its extent are profoundly influenced by the reaction of the tissue mixture. If the tissue is made alkaline or kept neutral, there is practically no evidence of autolysis, or it is at best an exceedingly small figure. If the tissue mixture is made acid, both the rate and the extent of autolysis are increased, as measured by amino-acids produced. It is certain that most of the substances studied which do accelerate autolysis, do so by virtue of their acidity. The increase in rate and the extent bear a rough proportionality at least to the amount of acid added, till an optimum concentration is attained This optimum is different for different acids, which indicates either that the H-ion influences the rate of autolysis or the negative ions have characteristic effects. At this reaction over 90 per cent of the liver proteins and those of other gland tissues appear as cleavage products. The insoluble residue is mainly connective tissue stroma. In the case of muscle the per cent of the total autolyzed is much less. The amino-acid curves of autolyzing acidified tissue are apparently identical with curves produced by adding some outside substratum, like gelatin or peptone.

Applying these generalizations to the problem of atrophy we may conclude that anything which tends to increase the acidity of a tissue *in vivo* will result in autolysis just as it does *in vitro.* Anything which maintains a tissue at its normal faintly alkaline reaction will prevent autolysis. When a tissue is alive and in place it does not atrophy unless it is injured directly, as by trauma, pressure, poisons, etc., or unless its blood supply is interfered with. In all such cases the normal metabolism is deranged, acidity develops, and atrophy results. Normal tissue has the same H-ion concentration as the blood, or pH = 7.4 ±. As long as this is maintained there is no evidence that autolysis can go on, either *in vitro* or *in vivo*.

H-ion changes. When a normal tissue is removed and examined at once it is found to be neutral or faintly alkaline to litmus. On standing it grows acid. Morse (75) studied the H-ion changes post mortem by the indicator method and found fairly rapid rise of the H-ion from about pH 7 to 6, which it approximates in 5 days and then remains constant. This reaction has been more exactly investigated by Dernby and also in this laboratory by the potentiometer method (76), (77). While it has not been possible to make determinations at the instant of removal, we have found soon after death a reaction of pH = 7 ±. Acidity develops rapidly and within 24 hours the H-ion of the mixture reaches the level of pH = 6.5 ±. For the next ten days the reaction drifts slowly back toward the neutral point as autolysis proceeds, usually reaching a level of pH 6.6 to 6.8. This may be referred to the gradual buffering of the acid by peptides and amino-acids, neutralization by ammonia, or to some other factor. After ten days the reaction slowly grows more acid again so that in twenty days it usually reaches a level of pH = 6.5 ±.

The rise in the H-ion concentration is explained by the production of organic acids such as lactic incident to the dying of the tissue and post-mortem changes. While considerable work has been done to determine the amount of acids produced and their variety, this phase of the autolytic machinery is still in need of further investigation. Lactic acid has been described and isolated by a number of workers, and it has been shown that leucocytes and tissues can form it from the hexoses in small amounts (78), (79), (80), (81), (82), (83), (84), (85), (86). It appears from H-ion measurements which we have made that added glucose has little measurable effect on the H-ion changes nor does glucose appear to alter the autolytic rate. Another source of acids is the fat of the tissue, and in the liver this is often considerable. We have found that such fats as cottonseed oil, cod-liver oil, olive oil, etc., lead to an increase of the H-ion concentration of an autolyzing liver brei to which they are added, and as they hydrolyze the fatty acids compete with the tissue proteins for the basic ions. The net result is that digestible fats in a liver brei increase its autolysis. Post-mortem development of carbon dioxide plays a part in the rise of acidity, but so small as to be almost negligible. It does however largely increaes the rate and extent of autolysis if added in sufficient amount.

The significance of the slow drift toward neutrality during the first ten days followed by an equally slow drift in the acid direction during subsequent periods is not understood. It is probably of no importance in actual atrophy where diffusible material is carried away. The initial acid production is in any event the determining factor in the extent of autolysis, and in those tissues where much acid develops autolysis is correspondingly large.

Two factors must be distinguished in the increase of autolysis by acid. One is the *total acid present*, and the other is the *H-ion concentration*. Both effect the reaction but in different ways. The enzyme effecting the primary cleavage of the tissue proteins ("Beta protease" of Hedin (87), the "endotrypsin" of Hahn (88), and the "pepsin" of Dernby (89)), is active only within certain H-ion limits and it has an optimum environment at which it catalyzes hydrolysis most rapidly. In the case of liver (pig) we have found the optimum reaction about $pH = 4.5 \pm$. This enzyme is probably quite inactive at $pH = 2.6$. These figures agree fairly well also with those reported by Dernby. As the H-ion concentration rises therefore, after removal of the tissue, the primary protease becomes more active, up to the optimum. At the same time the amount of acid developed in or added to a digest will determine the amount of amino-acids ultimately produced by determining the mass of substratum, and this will exert a mass effect in increasing the rate of amino-acid production up to the point where the enzyme is saturated with substratum. As acid is added to a digestive mixture, both factors are operative and it is difficult to differentiate the effect of one from the other. It can be done however experimentally by introducing equivalent amounts of acids of different dissociation constants. Thus in two digests to which the equivalent amounts of hydrochloric and acetic acids are added, we find much more rapid amino-acid production going on in the hydrochloric acid mixture. It has a higher H-ion level than the one with acetic acid present. In the course of ten days however the two digests have approximated equilibrium at the same level. The higher H-ion given by the mineral acid produces more rapid catalysis of the substratum available, but this mass is about the same in both digests, and this determines the final amino-acid production when equilibrium is attained.

In vivo the acids normal to metabolism are all such as to give relatively low H-ion concentrations. They are carbonic, lactic, butyric, with some sulphuric and phosphoric from the combustion of proteins and lipoids, and possibly other weak organic acids. Such acids would suffice to increase the mass of substratum, but the rate of its hydrolysis would not be very rapid. Under certain pathological intoxications mineral acids are produced in considerable amount, and in these instances autolysis is found to be both rapid and extensive.

The addition of acid quite evidently increases the mass of substratum for autolysis considered as a whole. This substratum we believe to be the acid-protein produced by the addition of acid to the base-protein salts of the normal tissue. This explanation seems all the more plausible when curves of autolyzing liver to which acid or gelatin are added, are compared. In both cases rate and amount of amino-acids produced increases in such a similar way that we are convinced the effect is actually the same—the effect of increased mass of substratum.

The effect of colloids. There is one series of observations that cannot be covered by this explanation. The extensive contributions of Ascoli, Izar and their co-workers (90) demand some special consideration, both because of the very large amount of data presented and because of its possible effect on the understanding of the subject as a whole. The first of a series of papers on the effect of colloidal sols on the autolytic reaction appeared in 1906. In this paper the metallic colloids of silver, gold and platinum were reported as increasing autolysis in a striking fashion. The three behaved alike qualitatively and almost quantitatively. The authors conclude that these metallic colloids catalyze the autolytic hydrolysis just as they catalyze the decomposition of hydrogen peroxide. These results seem to offer a reasonable explanation for the increased nitrogen catabolism resulting from injections of such metallic hydrosols (92), (93), (94), and silver salts (91).

In subsequent papers the same observation is extended to include the list of colloids previously enumerated The sols were stabilized with an amount of gelatin too small to produce a measurable effect on the amino-acid figures through its own digestion. They all increased autolysis from 50 to 100 per cent or even more. The inspiration of Bredig's classical work on the *Anorganische Fermente* (95) is unmistakable throughout the series, even to the confirmation of the "toxic" effect on these catalysts of such substances as phosphoric acid, mercuric cyanide, etc. It is difficult to escape the impression that much of this work was conducted with a definite mental bias which inevitably influenced the collection of data This impression is intensified by analysis of the data in the papers. For example, the addition of hydrochloric and phosphoric acid in amounts which regularly increase autolysis in the hands of other investigators failed to accelerate the process. This is entirely contrary to all the evidence of autolysis before or since. Furthermore we have repeated many of the typical experiments described, and are unable to confirm the results (96). It is true that certain colloidal preparations increase autolysis. In all cases which we have studied, the effect is due to acid, however. Commercial colloidal ferric hydroxide contains much ferric chloride which hydrolyzes to hydrochloric acid and produces a typical acid increase of autolysis When however this hydrosol is dialyzed for long periods until no further chloride can be removed, it is completely inert. As_2S_3 and Sb_2S_3 both accelerate autolysis, not because they are colloidal, however, but because in water they slowly decompose and give up H_2S, which accelerates autolysis. Sols of gold, platinum and silver have been prepared by the Bredig method of arcing under pure water, and by reducing reactions. But in no case have we observed the slightest effect on autolysis if all traces of acids are removed. We have reluctantly come to the conclusion that much of this voluminous contribution to the literature of autolysis is unreliable and so largely incorrect that it must be disregarded until it has been reinvestigated *in toto.* In later papers Izar and Truffi (97), (98) report that minute additions of silver and mercury salts will greatly increase autolysis. These results we have also repeated but are unable to confirm the observation

The autolytic enzymes. Jacoby described an enzyme of the liver as erepsin-like, since it would digest proteoses and peptones of autolyzing lung, but would not attack the lung tissue proper (99) Jacoby isolated the proteolytic enzymes of liver also by precipitating with ammonium sulphate. He believed them to be quite specific

Hedin (100) separated two enzymes from the spleen—"alpha protease" which digests in alkaline media, and "beta protease," digesting in acid media Buchner and Hahn described an "endotrypsin" which they obtained in the autolysis of yeast, resembling trypsin in its ability to carry protein cleavage to amino-acids, but differing from it in acting best in an acid medium (101)

Of the recent papers bearing on this question, the two by Karl Dernby (102) undoubtedly are of first importance For the first time the control of the H-ion concentration was perfected in this investigation. In the first paper Dernby presents the data obtained in a study of the autolyzing enzymes of yeast. It confirms the earlier suggestions of Hahn, Hedin and others, that there is present in yeast an enzyme which hydrolyzes the native protein to the peptone stage, acting best at about pH 4.5, a trypsin-like enzyme producing amino-acids from casein, gelatin and peptides and most active in a reaction of 7.0, and an ereptic-like enzyme, producing amino-acids from the peptides at a pH of 7.8, and having the specific property of the erepsin of the intestinal mucosa of splitting glycyl-glycine

The optimal reaction for total autolysis is about pH 6.0, or slightly on the acid side of neutrality At this point, as Dernby shows, the simultaneous action of the three enzymes is possible At a higher acidity peptic hydrolysis proceeds, but the tryptic and ereptic action is inhibited, while at about the neutral point the peptic action is arrested and the tryptic and ereptic cease for lack of substratum as soon as the preformed peptides are hydrolyzed The enzymes are studied in great detail, the effect of various added ions determined, the kinetics of the ereptic activity evaluated, and a mass of accurate data of the greatest value secured

In Dernby's second paper a similar study of tissue autolysis is made, and the results are interpreted in a similar way There seems however valid reason for questioning the interpretation. In effect the article describes the autolytic process as catalyzed by the three typical digestive enzymes pepsin, trypsin and erepsin Two of these names carry a very definite implication in regard to their properties They have applied for decades to the enzymes of the digestive tract, their optimal H-ion has been exactly measured, their products determined and the substrata on which they act. It is quite clear from Dernby's own results, and from our own (103), that the resemblances are not very close except in the case of erepsin and it is believed that the use of these historic names in this connection can only lead to confusion of ideas. It is not our intention to quibble over names, but in this case the application of the terms pepsin and trypsin, whose definition is clear from the research of decades, to enzymes which do not closely resemble in several important respects the originals, is questionable

The enzyme in liver which Dernby calls "pepsin" resembles gastric pepsin in facilitating the primary cleavage of the native tissue proteins made sufficiently acid. It probably does not produce free amino-acids in this cleavage any more rapidly than does gastric pepsin On the other hand, the liver enzyme has an optimal H-ion concentration of pH 4 5 while pepsin acts best at pH 1.5 ±. The liver enzyme is completely destroyed before this latter H-ion level is reached. This constitutes a very obvious and probably fundamental difference It can hardly be associated with the precipitation of some protein fraction of liver tissue and the consequent removal of the enzyme by adsorption in this accidental way, since pepsin added to a liver brei is not so destroyed but acts at its highest speed Pepsin is further characterized by the very wide variety of native proteins which it will hydrolyze under proper acidity. From some of our own unpublished experiments the primary protease of the tissues is more specific. Further work however is necessary before definite conclusions can be drawn in this particular. The apparent "activation" of this enzyme by acids may be due to the higher H-ion level, or it may be referable to the development of substratum in the tissue at an H-ion concentration above pH 7.0 or it may be and probably is due to both. It is clear at least that the base-protein salts which exist in the living tissue are not attacked by this enzyme, and the tissue as a whole therefore does not undergo autolysis. When acid is added, however, this is converted into free protein or acid-protein. At the same time the H-ion rises and the activity of the protease increases. Digestion of the acid-proteins proceeds, with primary cleavage producing peptides of a character which may then be further digested by the second enzyme complex, or erepsin. Autolysis as a whole is thus found to be roughly proportional to the acid added, and to the H-ion level also.

If the initial cleavage depended solely on the increasing *activity* of the primary protease in acid solutions, then it would appear that at a slight increase of H-ion level above pH 7 digestion of the tissues should be slow, but complete in time. This however is not the case. With each increment of acid, the total autolysis increases by a similar increment. The reaction goes rapidly at first and later reaches equilibrium. Equilibrium appears to be very definitely determined by the amount of substratum available, and this amount appears determined by the increment of acid. More or less of the tissue is digested, therefore, as more or less of it becomes dissociated by acids stronger than the proteins themselves.

The argument for the application of the name "trypsin" to one of the proteases found in liver or kidney is still less justifiable. Trypsin acts best between pH 7 and 8. It digests most of the native proteins and produces amino-acids as the end products. Complete cleavage proceeds so rapidly that there is little accumulation of proteose or peptone fractions in a typical tryptic digest. In the liver and kidney we find no enzyme which answers this description. The native tissue proteins appear undigested at pH 7 after months, and the amino-acid production is negligible. This is not due to any resistance of these tissue proteins to tryptic digestion at this H-ion level, for if trypsin be added to such a liver brei digestion goes on rapidly and completely, with the exception of the connective tissue residue. There seems to be no evidence therefore of a tryptic enzyme in the tissues which corresponds to the trypsin of the pancreatic juice and gland.

Erepsin, or the ereptases, on the other hand are widely distributed (104), (105), (106), (107). They are characterized by their inability to cleave the native proteins, casein excepted, but act upon the polypeptides, converting them into amino-acids. According to Abderhalden (108), there is an optimum H-ion concentration for the cleavage of each peptide, but the average is in the neighborhood of pH 7.8. Dernby has shown by the splitting of glycyl-glycine that a true erepsin exists in yeast and in such animal tissues as he examined. While it differs from the intestinal enzyme in its sensitiveness to various foreign ions, there seems to be no good reason for classifying it in a separate category.

While the tissue erepsin acts best at about pH 7.8, it is still active in as acid a medium as pH 3−. This is shown by adding a little pepsin to a liver digest made up to this H-ion level. Autolysis alone is completely inhibited at this acidity. Pepsin however effects the initial cleavage, and the erepsin present carries on the hydrolysis to the amino-acids rapidly. If the H-ion be raised to pH 1.2− the liver ereptase is completely inhibited, although the pepsin is very active. The amino-acids appear only at the very slow rate characteristic of the action of pepsin alone. In the living tissue the activity of erepsin is evidently conditioned by the production of primary cleavage products. These in turn are conditioned by the development of acidity within the cell. So long as the buffer mechanism within the cell maintains its normal reaction autolysis is completely in abeyance.

To summarize, we find that the enzyme mechanism of autolysis comprises at least two groups of proteolytic enzymes. One acts only in acid media and converts the acid tissue proteins into primary cleavage products. It is active between pH 7− and 2.6−, and its optimum is about 4.5. The second attacks only the primary cleavage products of the proteins, producing the amino-acids. It is active between pH 8− and 3, and seems to be identical with the erepsin of the intestinal tract.

The mechanism of atrophy. From the data presented above we may form what is probably a fairly accurate hypothesis for relating autolysis to the various normal and pathological atrophies. The normal tissue cell is maintained at the reaction of the blood, or pH 7.4. So long as its metabolic processes remain in equilibrium with its blood and lymph supply there is no accumulation of carbon dioxide, sulphuric acid, phosphoric acid or acids of intermediary metabolism. Neutralization of these acids, further oxidation of some of them, diffusion out of acid ions and diffusion in of basic ions goes on at such rates that the resultant reaction remains a constant.

Under these conditions the cell proteins are in the form of base-protein salts (109). The primary protease is inactive, the ereptase active but inoperative, and the amino-acids present in the cell are in equilibrium with the proteins and in diffusion equilibrium with the amino-acids of the blood. Any prolonged increase of metabolism within the cell without compensatory increase of the circulation about it would lead to an acidotic shift within the cell. Such shift, resulting from excessive acid production, leads to a rise in the H-ion and a change from base- to acid-protein. Primary cleavage results and the products are further disintegrated by the ereptase. The excess amino-acids diffuse out into the blood and lymph until an equilibrium is again attained. The net result of these steps is a cell of decreased mass and presumably of decreased metabolism, and the decrease continues until accurate adjustment between the cell and its blood supply is reëstablished. Any increase in acid production in cell or tissue beyond the capacity of the buffer mechanism to immediately dispose of must automatically tend to atrophic changes of mass.

We may apply this conception to a number of typical atrophies representing both normal and pathological conditions. The interpretations here offered are not all of them definitely proven. They represent however the field in which many of the future problems of autolysis must be worked out, and are therefore presented as tentative interpretations subject to change with new data.

1. The liver is particularly liable to intoxications and atrophic changes. Phosphorus poisoning leads to an acute atrophy in the course of which the entire liver is apparently destroyed and autolyzed. Phosphorus accumulates in the liver probably in the lipoid phase in which it is freely soluble. Like all strong reducing agents it produces asphyxial conditions in the cell which lead to acid development. In addition phosphoric acid is probably produced in the oxidation of the phosphorus. While phosphorus added to a stoppered liver digest does not increase autolysis, as Jacoby showed, it can be made to do so by passing a stream of air through the mixture. In any event the liver cells become markedly acidotic, die, and autolyze rapidly and completely.

2. In phosgene poisoning the alveolar lining of the lungs swells, disintegrates, sloughs off, and in general gives every indication of rapid and complete autolysis. Phosgene is a gas which is soluble in both lipoids and water. In the latter it is rapidly hydrolyzed thus:—$COCl_2 + H_2O = 2\ HCl + CO_2$. The gas penetrates the alveolar cells almost instantly and is as rapidly converted into hydrochloric acid. The cells die and begin autolyzing at exceptional speed on account of the high acidity developed within them. Chlorpicrin and other asphyxiating war gases owe their toxicity to this mechanism.

3. "Mustard gas" is perhaps the most striking example of a poison depending for its action on penetration followed by acid production. Dichlorethylsulphide is soluble in lipoids and fat solvents; it volatilizes slowly, is only slightly soluble in water, and hydrolyzes as follows: $(C_2H_4Cl)_2S + 2H_2O = 2\ HCl + (C_2H_4OH)_2S$. Marshall and his coworkers (110), (111), (112) have shown that "mustard" penetrates the skin by virtue of its organo-solubilities. Penetration is very rapid to the active dermal cells where the material concentrates in both the water and lipoid phases. Hydrolysis goes on slowly so that some hours after contact the developing hydrochloric acid starts the autolytic mechanism and eventually kills the cell. The rapid blistering and necrosis after the process first becomes evident is an expression of the speed with which the autolysis goes on under these conditions of excessive development of a strong acid within the cells themselves. In severe burns the whole area may digest and slough off, producing a lesion of marked chronicity.

The hydrolysis of "mustard" within the cell was definitely demonstrated by Lillie, Clowes and Chambers (113), who found that starfish eggs in water containing freshly dissolved gas absorbed some of it, and after a latent period during which development was normal, underwent necrotic changes and died. The products of "mustard" hydrolysis however had no effect on the developing eggs since they did not penetrate them. If the material was injected by micro-pipette into the cell there followed a similar latent period and then rapid degeneration. An equivalent amount of hydrochloric acid injected produced the necrotic changes of a similar grade at once.

In a further study of derivatives of "mustard" Marshall and Williams (114) found that those derivatives which had solubilities similar to "mustard," and on hydrolysis produced acids, were also the derivatives which were effective in producing skin burns.

4. The liver is subject to injury by toxins of biological origin (115), (116), (117), (118). Thus the injection of hemagglutinins, tetanus and diptheria toxins, streptococci and their decomposition products, has been found to injure the liver and lead to necrotic changes suggestive of some clinical conditions such as the liver in eclampsia or acute yellow atrophy. Jackson and Pearce studied the autolytic mechanism induced by injections of hemagglutinins. In certain cases the animals died early with the liver intensely congested and the capillaries plugged with thrombi of fused red cells. The animals which survived the immediate effects of the injection showed either a diffuse degeneration of the liver, or an organ dotted with focal necroses. The nitrogen output in the latter type was slightly elevated but normal in its ammonia-urea ratio. In the diffuse degenerating livers the ammonia-urea ratio was abnormally high. The authors interpret this as indicating that the hepatic function was obliterated in these animals while in those with focal necroses some cells were destroyed but enough normal tissue was left to carry on the hepatic functions. It is probable that there was a general acidotic effect in the diffuse degenerating cases which would also give a high ammonia-urea ratio. The authors believe there is evidence of protein storage in the early stages of intoxication, but in view of the intense congestion of the liver described it seems more likely that the increased protein represents the excess of blood (119). However this may be, the process as a whole illustrates the intoxication of the cells with altered metabolism, together with a reduced blood supply, tending toward asphyxial conditions, injury or death and consequent atrophic changes by the autolytic mechanism.

5. Acute yellow atrophy is a striking example of an idiopathic injury to the liver cells resulting in almost complete destruction and digestion of the liver tissue. In a case reported by Wells (120), the liver had shrunk to half the normal size before the death of the individual. In this case the parenchyma had autolyzed away while the stroma remained intact or perhaps proliferated to some extent. The liver was consequently much richer in gelatin-forming tissue than the normal. Connective tissues as a class do not autolyze and are rather resistant to digestion by enzymes from the leucocytes. In our own studies we have set up digests of mature cartilage and white fibrous tissue which showed no measureable increase of amino-acids after weeks in the thermostat.

6. Chloroform causes a more or less profound atrophy of the liver depending upon the length of exposure (121), (122), (123), (124), (125), (126). From non-fatal doses the organ recovers completely. According to Graham, the necrosis is caused by the break up of chlorform in the liver cells through phosgene to hydrochloric acid, and this would easily account for the autolytic changes which are found under way in such a liver. Graham has also shown that other halogen compounds similar to chloroform produce similar liver lesions and in proportion to the hydrochloric acid produced. Other chlor-compounds, like chloral, do not break up with the liberation of the halogen acid and these do not affect the liver. Iodoform and bromoform both cause liver degeneration, and the excretion of iodides and bromides in the urine makes it clear that the halogen acids are produced somewhere in the body. The liver lesions make it seem fairly certain that this decomposition takes place in that organ.

7. Atrophy of the mammary gland after lactation is a typical physiological example of autolysis resulting from diminution of the blood supply. Leaving the gland partly distended with milk hastens the atrophy through pressure, which cuts down the blood supply still more.

After parturition the mammary glands in some women produce an excessive secretion of milk, become tense with pressure and often painful. This great initial pressure regularly leads to a diminution through atrophy so that within a few days the supply of milk diminishes automatically. It is common practice in such cases to anticipate the excessive secretion by bandaging the breasts soon after delivery and thus initiating the atrophic changes and avoiding the over-production. It is also the practice to bind the breasts and leave them but partly emptied during the weaning period. In both these cases autolysis is initiated by pressure stasis and asphyxial cell-acidosis resulting from it.

8. Uranium salts produce nephritis with atrophic changes in the kidney cells. MacNider (127), (128) found that the administration of bicarbonate in considerable amount decreased the extent and severity of the lesions. It seems plausible that the excess alkali helped prevent the cell acidosis and so lessened the autolytic digestion.

9. Following parturition the uterus contracts, squeezes out the blood and lymph, and in the course of a few days or weeks atrophies to its resting size. The process is commonly hastened by administering ergot or some other drug inducing contraction in smooth muscle. The sustained contractions of this organ may be assumed to cut down blood supply. Carbon dioxide will be produced in increased amount, while it and the organic acids will be less effectively removed or neutralized. An organ-acidosis results which initiates and promotes autolysis, until the organ is again in equilibrium with its blood and lymph supply.

10. Contractions of the uterus are set up at term in some way not fully understood. There may be a number of factors which are operative in bringing on the expulsive contractions, the most probable are that they are started by some chemical hormone. It seems not improbable, at least, that autolysis may be an important factor. It is well known that protein cleavage products cause uterine muscle to contract (129), (130), (131), (132), (133). A certain threshold concentration is necessary before the organ responds. Any considerable separation of the placenta in late pregnancy must alter the blood supply to the separated area. If this separation is large enough the protein cleavage products produced in its autolysis would probably be sufficient to induce contractions. The settling of the fetus as term is approached, its more and more vigorous muscular movements, are both calculated to increase the strain on the adherent placenta. If a small separation only occurs it would perhaps fail to produce cleavage products in sufficient concentration to start active contractions. It might however account for the frequent preliminary contractions so often noted as term is approached. It is well known that a fall or a severe jar or even the mild shaking incident to an automobile ride is sufficient to start the process in many cases, and it is noteworthy that contractions do not begin at once, but after a latent period of some hours. This would correspond very well with the time required after such a separation to induce asphyxial development of acidity in the placenta and the production of the early cleavage products. It has long been known that the placenta contains proteolytic enzymes similar in character to those found in other tissues (134), and we have found that it behaves to acids and alkalies exactly like other tissues. At the optimum acidity the placenta will autolyze 400 per cent faster and farther than the control sample.

11. Voluntary muscle autolyzes slowly and incompletely. It is however influenced by the tissue reaction in the same sense as the more actively autolyzing gland structures (135), (136). An inactivated muscle gets less blood than an actively contracting one. Thus the arm hanging in a sling for some weeks shows considerable muscle atrophy. With this decreased mass goes a very large part of the contractile power of the muscle. When the muscle is again allowed to contract it gradually hypertrophies again to its normal mass and strength. *In vitro* experiments show that muscle autolyzes only about a quarter of its proteins under the most favorable conditions of reaction. The rest of the tissue is not hydrolyzed. It consists of connective tissues and muscle structural proteins which are not rendered available for autolytic cleavage even by acidity. It would appear that the protein fractions which are available material for the enzyme cleavage are particularly concerned with contraction, since removal of all or part of this fraction by atrophy *in vivo* removes at the same time practically all of the contractile power.

12. The atrophy of the tadpole's tail during the metamorphosis is an interesting example of the *complete* autolysis of a muscular organ. Gudernatsch (137) first showed that this atrophic phenomenon may be markedly accelerated by feeding thyroid material to the tadpole, and this has been confirmed by Morse (138), West (139) and others. It has been pointed out that the phagocytes play an important rôle in this process although Morse found no excessive number present in the tissues examined by him.

The explanation of the process is this: With the approach of the larva to maturity the bony structure known as the urostyle impinges more and more on the blood supply to the tail. Atrophic changes result Eventually cells die and the phagocytes invade the tissue adding their less specific enzymes to those of the tissue itself. As the blood supply diminishes slowly there is opportunity and time for the complete removal of the cleavage products as they are formed little by little throughout the tail. As is to be expected, there is no gross evidence of increased autolysis on testing the tissue. Even in the fulminating autolysis of the liver in acute phosphorus poisoning Jacoby found no accumulation of amino-acids until the circulation was lessened. Nor would it be expected that the tails would show an increased rate of autolysis *in vitro*. The asphyxial changes affect only a few outpost cells at a time, so that at any one moment there would be no gross increase of acid or of cleavage products in the tail as a whole.

The significance of the results of Gudernatsch lies in the increased rate of developmental changes resulting from thyroid, not in any specific effect which it, or other iodine compounds have upon autolysis (140), (141), (142), (143), (144), (145), (146). With rapid growth of the urostyle under thyroid feeding, the train of results depending on these changes must also go on at an accelerated rate.

13. Some epithelial tissues are particularly sensitive to x-ray radiation or exposure to radium. Such cells are injured in some way by the radiation, their metabolism is interfered with and they suffer atrophic changes. If rayed sufficiently they are destroyed. In the recent studies of Warren and Whipple (147) the great sensitiveness of the epithelium of the small intestine is described. The cells in the crypts appear more sensitive to the rays than at the tips of the villi. The microscopic picture of this tissue shows such very rapid disintegration of these cells as compared with the unrayed, that we should be inclined to suggest that autolysis was being reinforced by the enzymes of the digestive tract, trypsin and erepsin. If the cells are killed by the rays there is good reason to suppose their proteins will fix these proteolytic enzymes at once, while the protoplasm of the living cells does not have this property. Thus the dead cells carry with them to the digestion chamber enzymes in addition to the intracellular autolytic proteases, while the cells which were still living at the time the tissue was removed do not.

14. The brain is considered rather resistant to autolysis (148). This resistance is due however to its exceptional blood supply, its mechanism for increasing ventilation and blood flow to offset a developing acidotic shift within itself, and to the relatively small protein content of the brain. Of the proteins there too only a fraction becomes available in autolysis, the rest are structural and are not digested even under the best conditions. The brain behaves like other organs in relation to acidity and alkalinity (149), (150), and any asphyxial condition localized in some part of it through pressure, thrombosis, trauma, etc., results in digestion.

15. The softening of overgrowths and neoplasms is an example of autolysis induced by alterations in the blood supply. This may be caused by the contraction of freshly laid down fibrous tissue, so that capillary tufts are pinched off and their dependent tissues autolyzed.

16. In general acidosis and fevers the loss in tissue mass is correlated probably with acidotic changes within the tissue cells, leading to their partial autolysis.

17. The wastage in anemias seems also to be related to the decreased oxygen supplied the tissues with partial asphyxia and autolysis toward a new equilibrium between the mass and metabolism of the tissues of the body and their supporting blood and oxygen supply.

18. In starvation we have a striking example of slow autolysis by which a very large portion of the proteins of the body is digested and thus mobilized for the use of the more essential organs. It is evident that the tissues least subject to autolysis in starvation are those whose blood supply is most assured, the brain and heart (152). This slow autolysis is probably related to blood supply and tissue acidosis as in the other cases mentioned. It is certainly not a simple activation phenomenon as described by Schryver (151), although activation by increased H-ion probably plays a part. Generalized acidosis is a prominent and persistent feature in starvation and it is fair to assume that it is expressive of conditions within the tissue cells. With loss of muscle proteins muscular weakness slowly increases, the individual tends to become more and more quiet. Decreased metabolism, decreased blood supply, lowered blood and pulse pressures are factors which must automatically lead to conditions favorable to autolysis (153). Thus the essential structures, like brain and heart, survive at the expense of those less perfectly supplied with blood. The skeleton persists also with very little loss because, along with the other connective tissue, it does not autolyze.

19. The very slow generalized atrophies of advancing age are probably also related immediately to a blood supply growing gradually less efficient, and leading to a concomitant decrease in the mass of tissues maintained in equilibrium with it.

These are but a few of the more obvious examples of physiological and pathological processes in which autolysis plays a rôle. Every loss of tissue mass which is not merely due to loss of water, involves this mechanism. It is safe to assume that every increase of mass, as in hypertrophy and growth, involves the same mechanism operating in the reverse direction. The factors involved in growth and hypertrophy are so much more complicated than in hydrolysis of a tissue that we know little of it experimentally. It is obvious that it requires an abundant blood supply, and a reaction which insures the stability of the base-protein complexes. The interesting observation of Menten's that in cases of carcinoma the pH of the blood is about 7.8 is suggestive (154). So too are the results obtained by Fischer in growing fibroblasts in culture media (155). The optimum reaction for growth was between 7.4 and 7.8 and the cells were more resistant to abnormally high alkalinity than to acidity. Moore, Alexander, Kelly and Roaf (156) found that fertilized sea urchin eggs are never stimulated to growth by increasing the acidity of the surrounding medium, but that they are stimulated by slight extra alkalinity, and may develop atypical mitotic figures which are similar to those seen in malignant tumors.

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THE ORIGIN AND SIGNIFICANCE OF THE CONSTITUENTS OF THE BILE

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In attempting a review of the many constituents of the bile it is obviously impossible for the writer to include all the contributions in this field. If some important papers have received too little attention the writer hastens to apologize. It is always difficult for a reviewer to evaluate with judicial fairness the experimental data of other investigators, especially if he has been concerned with experiments which are not in accord with those under review. No review concerned with bile constituents would be complete without reference to Stadelmann's monograph, *Der Icterus* (51). It would be well if all investigators in this field were to read this book from cover to cover, for the procedure might conserve to the research journals much valuable space. Stadelmann's reviews and his experiments are models which must excite admiration in all students of this subject. It may perhaps be convenient to review each important constituent of the bile before noting certain facts concerning the whole bile.

BILE PIGMENTS The bile pigments may or may not be the most important constituents of the normal bile but certain it is that they have received the largest amount of attention, clinical and experimental. Bile pigments appear in demonstrable amounts in the bile canaliculi of the normal or abnormal hepatic epithelial cell. It is therefore a bit too easy to think of the hepatic cell as the only essential factor in the elaboration of bile pigment. But it is well for physician, teacher, student and investigator to keep clearly in mind that *other body cells have the capacity rapidly to change hemoglobin to bile pigment.*

Whipple and Hooper (57) showed that this transformation could be brought about within 2 hours in the blood stream of the head and thorax with complete liver exclusion. The same workers (18) showed that hemoglobin can be transformed into bile pigments within the serous cavities during a period of 12 hours or longer. McNee (31) and Van den Bergh and Snapper (54) have confirmed a part of this work. It is highly probable that the vascular endothelium and serous mesothelium are concerned in these reactions. We wish to emphasize the point that *these reactions* in our opinion *are not physiological curiosities* but concern the normal bile pigment production. It seems probable to the writer that the Kupffer cells are concerned in the normal production of bile pigments and it is not a mere accident that these cells make up a considerable part of the gross liver tissue in the most intimate association with the hepatic parenchyma cells. It would seem possible that the formation of bile pigments might be a *function of both the liver endothelium* (Kupffer cells) and the *hepatic epithelium*, the preponderance of activity being determined by factors not understood at present. It is suggested that the Kupffer cells may be concerned with production of bile pigments from hemoglobin present in the blood stream while the hepatic epithelium may be especially concerned with the manufacture of bile pigments from other substances which (pigment complex fig. C) may be derived from the food or body cell protoplasm.

Our conception of the relations of bile pigment to other body pigments is illustrated in a crude way in figure C. Many important factors concerning the interrelation of hemoglobin, bile pigment and other body pigments have been reviewed by Whipple (56), and we must refer to that paper for much of the detailed discussion of pigment metabolism. Those interested in the chemical structure of bile pigments are referred to papers by Küster (22), (23), (24).

It has been claimed by some investigators (Brugsch, Yoshimoto, Kawashima (3)) that hemoglobin introduced into the blood stream will be quantitatively excreted as bilirubin in the bile. Whipple and Hooper (61) have been able to show that in their bile fistula dogs no such quantitative relationship holds for hemoglobin and bile pigment. It seems very probable that much of the hemoglobin set free in the blood stream may be used in the body economy for a variety of purposes—among others, the construction of hemoglobin for new red cells. For example, it is sufficiently well established that intravenous injections of hemoglobin or the destruction of red cells in the body will aid in the recovery from simple anemia with consequent upbuilding of new hemoglobin (20), (21). It is very probable, however, that the hemoglobin in the blood stream is not used directly but only after being broken down to the unit structural factors—whatever these may be. It may be difficult or almost impossible to prove beyond a doubt that hemoglobin may not be quantitatively eliminated as bile pigments in the bile but it is relatively easy to show (17) that hemoglobin introduced intravenously, intraperitoneally or intramuscularly will aid materially in the recovery to normal from a simple anemia. If the introduced hemoglobin may be used to form new hemoglobin it is scarcely permissible to state that it is quantitatively eliminated as bile pigment. This point is illustrated graphically by double arrows between the *pigment complex* and *hemoglobin* in figure C.

It is well established (58) that the bile pigment elimination in bile fistula dogs can be increased by a change in diet—for example, a sudden change from a meat to a carbohydrate diet may increase the bile pigment elimination more than 50 per cent. This can be repeated time after time and it seems at least improbable that this reaction is dependent upon blood destruction. We may wish to explain this reaction in part as follows: The meat diet is normal for the dog and the increase in bile pigment excretion due to carbohydrate excess may represent an abnormal or alternative reaction—a deviation of pigment elements and construction into bile pigment for elimination. It is possible that some of these pigment elements concerned in this reaction might be available under favorable conditions (anemia) for hemoglobin construction or under usual conditions (meat diet) for elimination

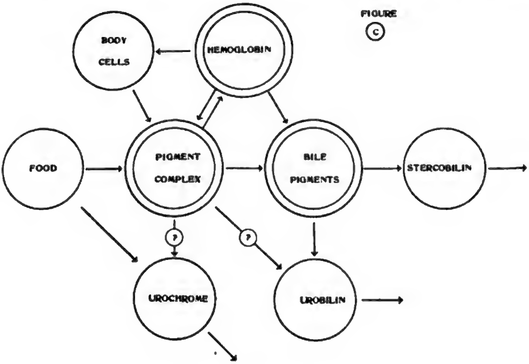

FIG. C

elsewhere than in the bile. Urochrome is a possible end product of pigment elements and deserves much more study in normal and abnormal conditions.

The term "pigment complex" is used in figure C to indicate a group of substances which are essential parts of mature body pigments. It is obvious that certain food factors contribute to this "pigment complex," as food is directly concerned in the production of new hemoglobin and the formation of bile pigments and urochrome. It is equally clear that the body protein and cells contribute to this "pigment complex" as all body pigments are produced in measurable amounts during fasting periods. We believe the evidence is sufficient to show that hemoglobin as it disintegrates in the body also contributes to the "pigment complex" and so influences in a measure the new formation of hemoglobin. It is probable that only a small amount of the destroyed hemoglobin is conserved in this fashion. The pyrrol nucleus seems to be one of the factors which must be concerned in this "pigment complex" and it is probable that all facts related to pyrrol metabolism will have a direct relation to the complicated body pigment metabolism. The above points are illustrated graphically in figure C.

Bile pigments in the bile fistula animal are not increased by the feeding of fresh bile pigments or of fresh or cooked blood or of digestion products obtained from blood (59). This might be assumed to be from lack of absorption. There is no evidence that bile pigment or stercobilin are absorbed from the intestine. However, it has been shown that the feeding of hemoglobin will influence the curve of new hemoglobin construction after anemia (17). This indicates an absorption of substances which are concerned with "pigment complex" but it is clear that these same factors do not influence the output of bile pigments in the bile fistula dogs—at least under the conditions of our experiments. These experiments are much against the interesting suggestion of Wilbur and Addis (64) that there may be a conservation of bile pigment factors which are absorbed from the intestine and reconstructed into hemoglobin. Perhaps the strongest argument against the absorption of stercobilin and its utilization in body pigment construction is the fact that bile fistula dogs under observation continuously for 2 years or longer show no evidence of pigment lack, no anemia, no fall in pigment production and no reaction whatever to the feeding of bile pigments.

A study of the *bile pigment output of the Eck fistula liver* furnishes some interesting facts to consider at this time. Dogs with combined

Eck and bile fistulae eliminate less bile pigment than controls—sometimes but 30 to 50 per cent of normal (60). The Eck fistula liver is functionally inefficient and there is no direct contact with the portal blood. Both these factors may well contribute to this low pigment output but the main point to emphasize is that the pigment output is influenced by *liver function* rather than by the amount of hemoglobin waste products formed in the body. We have ample evidence that various liver injuries will likewise depress bile pigment excretion—again clear evidence that the liver has a constructive function in producing bile pigments rather than a simple passive eliminative function.

Certain conditions in bile fistula dogs will cause or be associated with a maximal bile pigment elimination—for example, a combination of splenectomy and anemia (19). Such periods of maximal bile pigment formation are of very great interest and deserve careful study as such investigations may lead to a more complete understanding of the *fundamental pigment stimulus* in the body. This is of obvious interest to physiologist and clinician alike.

One last point concerning pigment formation invites speculation. We recall that vessel endothelium (including Kupffer cells) and mesothelium (lining cells of serous cavities) can rapidly transform hemoglobin into bile pigment. Further, the embryo endothelium in the chick is able to develop red blood cells containing hemoglobin (47). We recall the embryonic hematopoietic function of the liver containing hepatic epithelium and Kupffer cells. We have pointed out the intimate relationship between the two types of cell normally concerned with bile pigment production (hepatic epithelium and Kupffer cells). The degradation of hemoglobin to bile pigment concerns two important cells (liver epithelium and Kupffer cells). May these two cells have any capacity for reversing this reaction and may they not be concerned with hemoglobin construction or the production of parent substances used by the marrow cells which put the finishing touches on the hemoglobin of the erythrocytes? There are many suggestive things in animal experiments but convincing proof of this point is not yet at hand although at times it seems to be almost within one's grasp.

UROBILIN. It may be debatable as to whether urobilin is a normal constituent of bile. However, it is claimed by some investigators that it is present in normal human bile (53). These claims are based on analyses of bile obtained by the duodenal tube and obvious objections may be raised to these conclusions as the bile was in contact for a short time at least with the intestinal mucosa and its intestinal flora. Urobilin is constantly present in fasting bile-fistula dog bile (62). Urobilin has been observed frequently in human bile under abnormal conditions (Wilbur and Addis (64) and others). It has been suggested by Brulé and Garban (4) that urobilin is formed directly from the hemoglobin by a reaction which goes on in the circulating blood and living tisues. However they give no experiments to support this very interesting suggestion as it is obviously necessary to exclude the participation of the liver. Quadri (41) submits a series of experiments to show that *in vitro* the common bacteria cannot change hemoglobin into urobilin.

We must refer to a fundamental error which is unfortunately firmly fixed in the minds of clinicians and laboratory workers—the belief that stercobilin or urobilin is absorbed from the intestine. We know of no evidence of any sort to indicate that urobilin or bile pigments are absorbed from the intestine, once we grant that urobilin may be present at times in the bile within the bile ducts. We believe that urobilin is formed in the liver, particularly in abnormal conditions of the biliary tract (cholangitis) but know of no evidence that it is absorbed from the intestine—refer to figure C.

All the evidence at hand indicates that the pigment substances in the bile (bilirubin, biliverdin and urobilin) subsequently serve no useful purpose and are true excretory substances. There is no evidence for any "circulation" of the bile pigments and when this word is used it should be limited to the bile salts which are so rapidly absorbed from the intestine.

BILE SALTS. In this discussion we may concern ourselves with glycocholic (including glycocholeic) and taurocholic (including taurocholeic) acids which are found in the bile as the sodium salts. If our ignorance about the complete story of the bile pigments is disturbing, then our lack of understanding as to the source and internal metabolism of the bile acids is pathetic—this in spite of much careful study and investigation. The most important fact to date is the "circulation" of the bile acids; by which we mean a rapid absorption of the bile salts from the intestine with prompt reappearance in the bile. This fact was established very early in the study of bile physiology (49) yet we understand nothing as to the control of this reaction in the body. We may be led into a ridiculous position if we choose to argue from the few facts established by experimental work—for example, a bile-fistula dog excretes considerable amounts of bile acid during fasting periods, therefore the substance is produced constantly in the body. Again we find good evidence that bile acids by mouth in bile fistula dogs are promptly and quantitatively eliminated in the fistula-bile—for example, 1 gram bile salts by mouth will be *excreted quantitatively in the fistula bile within 6 hours.* In the normal dog whose bile escapes into the duodenum we should indeed find an amusing condition—new bile acids formed daily and all the bile acids poured into the intestine *quantitatively* absorbed—a vicious circle with rapid transformation of the dog into a pillar of bile salt! Evidently there is a mechanism in the normal animal which controls the production and destruction of bile salts within certain limits but this is a complete mystery.

One great difficulty was the lack of accurate and rapid methods for bile acid analysis and the older work required large amounts of bile, much time and expensive chemical extractives. The new method of Foster and Hooper (12) is a great improvement and permits of rapid and accurate analysis of taurocholic acid in small amounts of dogs' bile. They give a critical review of the older methods. Their method consists of hydrolysis of taurocholic acids into cholic acid and taurin. The amino nitrogen of the taurin is then determined by the gasometric method of Van Slyke. A further modification of this method by Schmidt and Dart (48) enables the investigator to determine accurately the two separate bile acids, taurocholic and glycocholic. They find complete absence of glycocholic acid in dog and sheep bile, a preponderance of glycocholic in pig's bile and approximately an equal division between the two acids in human fistula bile.

We may now ask what information has been obtained from fistula animals as to bile acid production. For a detailed discussion of these factors we wish to refer to the papers by Foster, Hooper and Whipple (13). Bile fistula dogs are not wholly satisfactory but are the best experimental animal at present and most of the accurate information is obtained from observations on these dogs.

Under normal conditions of health there is great variation day by day in the output of bile salts. A part of this variation is due to diet factors, and in general the bile salt excretion is much greater on a meat diet than on a carbohydrate diet. The actual curve of bile acid secretion as related to food intake is not easy to determine because of factors other than food which influence bile acid production. For example, it has been noted repeatedly that after a fasting period of 2 to 4 days or longer the prompt rise in bile acid production following a meat feeding may not appear until after the second or third feeding. This suggests a fasting depletion of a reserve material which is made up before the customary rise in bile acids appears after meat feeding.

During fasting periods there is a uniform excretion of taurocholic acid in bile fistula dogs. This level of excretion will be lowered somewhat by sugar feeding and we note an interesting parallel between the bile acid excretion and the urinary nitrogen excretion. Of course this suggests an important relationship between body protein metabolism and bile acid metabolism. Evidently there is an important *endogenous factor in bile acid metabolism.*

Within limits, an increase in food protein will reflect an increase in bile acid excretion. The level of bile acid excretion can be raised to a maximum by meat feeding (exclusive of bile salt feeding). This fact suggests that there are important *exogenous factors in bile acid metabolism.*

The Eck fistula liver furnishes us with very important data: This type of liver is known to be functionally subnormal and it produces about one-half the normal amount of bile acid on a standard diet. This is important evidence that the production of bile acids depends at least in part upon the functional capacity of the hepatic epithelium. It is generally accepted that bile acids are formed only by hepatic epithelium but the evidence for this is fragmentary and, when critically reviewed, none too convincing. The Eck fistula liver furnishes evidence to support the accepted belief and other experiments will be published soon to give further confirmation. These experiments (Smyth and Whipple) will show a remarkable depression of bile salt excretion due to *small doses* of poisons known to act specifically upon hepatic epithelium.

Bile acids can be readily separated into their constituent parts: taurochloric acid = taurin + cholic acid and glycocholic acid = glycocoll + cholic acid. Both these animo acids are present in the body and glycocoll can be formed within the body. Taurin is derived probably in large part from the cystein of the food or body protein. Under usual diet conditions there is probably an excess of glycocoll and taurin available in the body to satisfy any demands made in the bile acid synthesis.

Cholic acid is the determining factor in this reaction and we may as well begin with the statement that almost nothing is known about the origin of cholic acid in the body and the disposal of any excess of this compound other than in the bile. Cholic acid has a structural formula which is rather complex but contains no nitrogen. Pregl (40) states that cholic acid is a hexahydroxybenzene and structurally related to turpentine and camphor. A considerable literature on this subject has been recently reviewed by Foster, Hooper and Whipple (14). In that paper sufficient evidence is submitted to prove that no physiological relationship exists between camphor and turpentine and cholic acid.

The same holds for cholesterol which has often been suspected as the precursor of cholic acid (2), (65), but careful experiments negative this interesting suggestion. In the same series of experiments (14) it was shown that red cells fed by mouth or hemolyzed and introduced intravenously had no influence on the bile acid excretion. A recent paper by Beth (2) reviews the suggestion that bile acids are derived by the disintegration of cholesterol. He claims that this interesting reaction takes place within the Kupffer cells but we were unable to locate the data to establish this claim. Further objection to this work may be made on the grounds that the material is obtained by the duodenal tube. One must be guarded in the study of this material as analyses can be at best qualitative, as we are dealing with unknown dilutions and confusing additions of gastric and duodenal secretions. We may conclude that *cholic acid is the limiting factor which determines the level of bile acid excretion in fistula bile but the origin and fate of cholic acid are obscure.*

In this connection we may note that taurin intravenously has no effect on the excretion of bile acids(14). Taurin plus cholic acid by mouth causes a notable cholagogue action and increase in bile acid output just as taurocholic acid by mouth. Evidently these substances have a strong physiological attraction within the body for we note the same effect if the cholic acid is given by mouth and the taurin intravenously. Cholic acid fed during long fasting periods gives a minimal output of bile acids but fed during full diet periods gives a maximal output of bile acids with the usual cholagogue action. We believe this reaction is to be explained by the available supply of taurin within the body which is at a low level during fasting but not during full diet periods.

The bile acids are generally believed to be formed only by the activity of the hepatic epithelium, as stated above. These substances are looked upon as true secretory factors as they serve a useful purpose in the digestive process within the intestine. We recognize a significant influence exerted by the bile acids upon fat digestion and intestinal putrefaction. It would be of great interest to learn whether the bile acids are concerned with any of the endogenous fat metabolism reactions which take place within the liver. There are scores of interesting possibilities connected with bile acid metabolism but space does not permit such discussion.

DISSOCIATION OF BILE CONSTITUENTS. Stadelmann was the first to call attention to this interesting fact. He emphasized the versatility of the hepatic epithelial cell. It is very easy to give examples in which one or the other of the bile constituents may be increased without equivalent increase in other constituents. Hemoglobin given intravenously will cause a rise in bile pigments but no significant change in bile salts. Taurocholic acid by mouth will cause a great increase in total bile flow and bile acid output but the total bile pigment may be decreased. Taurocholic acid plus sugar by mouth may show no increase in volume but great increase in bile acids. This experiment probably shows the maximum power of the liver to concentrate bile acids in bile and the concentration may rise to 7 or 9 per cent by weight. The water elimination in the bile is obviously influenced profoundly by sugar or carbohydrate feeding. Meat feeding will be associated with a high bile acid output and a lowered bile pigment excretion. Instances need not be further multiplied. Much less is known about the fluctuations of the other bile constituents and such data are very much to be desired.

CHOLAGOGUES. The chief if not the only real cholagogue is *whole bile* or its active principle—that is, the *bile acid* fraction. The mere fact that drugs are claimed to be cholagogues by some and denied by others will indicate to the student of bile physiology that the cholagogue action is at least insignificant if not absent. We may note the few points about which all workers are in accord. Bile salts by mouth or intravenously will cause a distinct rise in the volume of bile secreted by a temporary or permanent fistula. Likewise a meat diet favors an abundant flow of bile in a bile fistula dog but a diet rich in carbohydrates, especially sugar, will decrease the bile flow under similar conditions. That *sugar has a distinct inhibiting influence* on the bile flow can be shown by combining bile salts and sugar by mouth. This procedure will give a maximal concentration of bile acids in the bile but little or no increase in the volume of bile. This shows the importance of diet as influencing the bile flow.

The duration of the cholagogue effect of bile salts depends upon the dosage. With a dose of 1 to 2 grams taurocholic acid the cholagogue action will be over 4 within 4 or 8 hours and the ingested acid can be recovered quantitatively in the fistula bile. Larger doses of taurocholic acid (8 to 12 gms.) by mouth may prolong the cholagogue effect for 24 to 48 hours or even longer. It is generally admitted that glycocholic acid has a less powerful chologogue effect than has taurocholic acid.

What shall we say of the various drugs advocated as cholagogues by certain investigators? We believe the attitude of Stadelmann is correct—that one must be critical of claims not supported by uniform and distinct increases in bile flow such as may be produced by bile feeding. None of the so-called cholagogues stand this test except, as stated above, the bile salts (especially taurocholic acid). The salicylates have many advocates as cholagogues (25), (37) and as many report negative results. The writer has never observed any constant reaction to salicylates in bile fistula dogs. Neubauer (36) and others have studied the effect of pilocarpin, adrenalin and atropin. We find no uniformity of reaction or opinion. The changes noted are not striking and may or may not be due to the action of the drug on the liver epithelium. Large doses of adrenalin are reported (36) to cause fall in volume excretion. The same indefinite reactions are reported by Weinberg (55), Okada (37) and others following administration of dilute acids, soaps, salts, glycerine and albumoses. Downs and Eddy (7), (8) report minor fluctuations in bile flow due to a host of organ extracts, adrenalin, secretin, etc. Eiger (10) claims to demonstrate secretory fibers in the vagus which influence bile flow from the liver. The changes are minimal and the writer is not convinced that the changes can not all be explained on the basis of simple vasomotor reactions.

We have considerable evidence that certain drugs can relax the bile papilla sphincter and perhaps also cause certain constriction of the bile ducts and gall bladder. For example, Doyon (9) showed that duodenal irritation might cause relaxation of bile papilla and constriction of gall bladder and ducts. Meltzer and Auer (32) emphasized this reaction as caused by magnesium sulfate and recently this has been applied to the clinical study of disease (30), (50). Much information of value is to be expected from a careful study of normal and diseased humans by this method. There is need, however, for critical analysis of these data to guard against too free interpretation of results.

All physiologists who have worked with bile fistula animals under anesthesia or shortly after an operative procedure are all too familiar with periods of temporary inhibition of bile flow. Such periods may last for hours and seriously interfere with the experimental procedure. The cause for this is not understood although it is believed by many that operative manipulation of the gastro-hepatic omentum and nearby structures will predispose to such a condition. Whether there is such a thing as spasm of the smaller bile ducts or actual reflex inhibition of the hepatic epithelial secretion must for the present be left an open question.

In conclusion we may repeat that the only active cholagogues are bile or bile salts. A number of drugs are proposed as cholagogues by a host of investigators but when we examine the experimental data we find minimal fluctuations caused by a variety of drugs—salicylates, chloral hydrate, soaps, acids, albumoses, etc., etc. If cholagogue action is present it is slight, or delayed or transient and is usually less than can be demonstrated as due to food factors (meat, for example). Such cholagogue reactions fade by comparison with the reaction caused promptly by bile salts. This reaction is constant and lasting and of striking volume. When claims are made that a substance is a cholagogue it should always be measured by the standard of the only known active cholagogue (bile salt) and to date all such substances fail when so tested.

CHOLESTEROL, PHOSPHATIDS, FATS, SOAPS: *Cholesterol* appears to be the most important member of this group and this is to be explained in part by the fact that cholesterol is such an important constituent of gall stones. The formation of biliary calculi is of much concern to physiologist, pathologist and surgeon. Experimental work with cholesterol will be greatly furthered by an accurate, rapid and simple analytical method but as yet this method is not available. The bile acids, bile pigments and lecithin are sources of trouble in adapting methods to cholesterol analysis in bile.

Suggestions as to the source of cholesterol may not as yet have exhausted all the possibilities but their number is legion and the writer will mention but a few. Stadelmann (51) suggests its origin from the bile duct epithelium and degenerating liver cells. He could find no evidence for increase in the bile following cholesterol feeding. On the contrary, Fasiani (11) reports an increase in bile cholesterol in bile fistula dogs after intravenous injection of large amounts of cholesterol. It is fair to say that the rise is only from an average of 5 mgm. per day to about 13 mgm. per day after a dose of 1.7 grams intravenously. It is obvious that only a very small part of the cholesterol so given is excreted promptly in the bile. Fasiani claims an important relationship between the blood and bile cholesterol—any excess in the blood being excreted in the bile. We cannot see the evidence to establish this point.

Feeding experiments by D'Amato (6) give similar results but he uses ox brain and eggs in the food to give the excess of cholesterol—about 5 to 6 grams cholesterol per day. This great excess in the food increases the bile cholesterol from 6 mgm. to 8 mgm. per day and he states that bile is evidently not the chief avenue of elimination for cholesterol in the food. Stepp (52) placed rats and dogs on a lipoid-free diet for a number of weeks and records a fall in bile cholesterol to 10 or 15 per cent of normal found at autopsy. It may be objected that these very abnormal diets caused systemic abnormalities which were responsible for this low cholesterol level rather than the immediate lack of cholesterol in the food. Some very suggestive experiments are recorded by Gardner and Fox (15). They report in man on standard diets an *excess of excretion over cholesterol intake*. Cholesterol is obviously synthesized in the body if these observations are correct and this work should stimulate further investigation in this field. It would be very desirable to know whether this excess excretion is present both on diets poor in cholesterol as well as on diets rich in cholesterol.

Among others Chauffard, Laroche and Grigaut (5) have suggested control of cholesterol metabolism by the adrenals. They believe that the adrenals are the sources of cholesterol and control the body supply. It would be somewhat difficult to disprove this possibility but the writer can find no proof furnished by the authors that the adrenals control cholesterol metabolism and the proof should come from the proponents of this hypothesis. Endocrine control of every body function may be a fact but much of the present enthusiasm for such explanation unfortunately has little or no ballast of solid fact. This type of ballast is sadly needed when one embarks on the uncharted sea of endocrinology.

We find repeatedly the statement that cholesterol is the precursor of the bile salts, as noted above. Lifschutz (28) goes so far as to claim that bile acids are derived quantitatively from cholesterol. Such claims are usuallly made by chemists who are impressed by the close chemical relationship which is known to exist between cholesterol and the bile acids. Windaus (65) for example shows that an identical split product can be produced by hydrolysis of the two substances. Physiological experiments do not support this claim and in fact exclude the possibility that bile salts in body metabolism are derived from cholesterol. The experiments of D'Amato (6) and Foster, Hooper and Whipple (14) are convincing.

The cholesterol content of human bile is given by Nathan (35) and Rosenbloom (44). There are very wide variations both in health and disease. Human fistula bile contains 0.07 to 0.10 per cent cholesterol. Human bile at autopsy is very different in content and may vary all the way from 0.06 to 1.06 per cent. The variations are as great in cases with normal liver findings as in cases with liver and biliary tract disease. In the interpretation of analyses of gall bladder bile one must not forget the remarkable concentrating power of the gall bladder, therefore casual analyses of such bile can have relatively little significance as to quantitative values. Cholesterol excretion has been studied by means of the duodenal tube but there are serious limitations to this method when applied to quantitative analysis.

To sum up, we may give the few facts which relate to cholesterol metabolism. Lipoid-free diets may be associated with low bile cholesterol values. Excess feeding of cholesterol gives but trivial increases in bile cholesterol. The elimination of cholesterol in man may exceed the cholesterol intake, indicating a production of this substance in the body. The cholesterol content of human bile varies within wide limits and one can determine no uniform relationship to disease. It is possible that diabetes and pregnancy are exceptions to this statement. The evidence is very strong that there is no physiological relationship between cholesterol and bile acids. It is suggested by various writers that cholesterol results from secretion of the biliary tract epithelium, from liver parenchyma degeneration, from red cell disintegration, from general tissue wear and tear, from food cholesterol, from the adrenals and other glands of internal secretion, etc. Nobody has as yet suggested that cholesterol is derived from the wear and tear of the cerebral cortex under the stress of environmental conditions!

Lecithin has been found in specimens of bile analyzed by chemists. This applies to human and animal bile. Rosenbloom (44) gives figures for many such analyses. Lecithin is reported usually as less in percentage content of the bile than given for cholesterol but Rosenbloom finds more lecethin than cholesterol. The values given for human bile vary from 0.05 to 6.4 grams per 1000 cc. bile. The bile of herbivora appears to contain less lecithin than does human bile (43). Extraction and analysis of lecithin in bile is very difficult. Long and Gephart (29) have emphasized the tenacious union which exists between bile acids and lecithin and it is very difficult to separate these two substances by extraction methods.

Jecorin has been found in bile but recent work of Levene indicates that this substance is a mixture of two phosphatides, lecithin and cephalin. Levene (27) reviews evidence that lecithin from the liver is different structurally from egg lecithin but this liver lecithin may or may not be identical with the bile lecithin. The writer perhaps has made it clear that there is plenty of work yet to be done before an understanding of the origin and significance of lecithin in the bile may be approximated.

Soaps and fats are always reported as present in chemical analyses of bile. In many analyses we find the ether extractions lumped together. We know of no experimental observations to give information as to normal or abnormal fluctuations in the fat or soap content of the bile. Few if any suggestions as to the source of such substances have been made by the workers in this field. We may conclude that our ignorance on this subject is approximately 100 per cent.

MINERAL CONSTITUENTS. So far as is known, the salts are mainly chlorides of sodium and potassium, and phosphates of calcium, magnesium and iron. There are traces of copper and traces or no sulphates. Inorganic substances in human bile are given as 0.6 to 1.1 per cent, but concentrated bladder bile may be much higher in salt content.

Iron elimination in the bile has been much studied and it is fair to say that there are wide differences of opinion. Some workers deny the presence of iron in bile but they are in the minority. Some workers claim that iron elimination is much influenced by food iron intake. Leone (26) claims that in bile fistula dogs the normal level of iron secretion (4.2 to 8.2 mgm. per 100 cc. bile) can be raised on a diet rich in iron in protein combination to a level of 22.8 mgm. per 100 cc. bile. He finds less increase on subcutaneous administration and a negative reaction on giving inorganic iron by mouth. The body metabolism of iron is certainly not completely understood and information is very much to be desired. A study of the causes of fluctuation of iron excretion in the bile will surely give information of much value.

Excretion of the *chlorides* and *phosphates* in the bile has been very little studied but there is evidence that these salts in the bile have some significance. We may give an illustration. Bile fistula dogs under observation for many months very often develop *bony abnormalities*. The essential feature of this condition appears to be a loss of inorganic salts from the bones with a great thinning of all bones, and spontaneous fractures of ribs and long bones. This indicates a great loss of salts from the body presumably in part through the bile and a lack of power of assimilation. We hope to report the complete mineral metabolism of these dogs in the near future. Another of the many curious factors concerned in this condition is the observation that the addition of cooked liver to the usual diet will prevent the development of this condition. The main point we wish to make is that the bile excretion in bile fistula dogs may be concerned with the mineral metabolism of the body and bony framework.

MISCELLANEOUS. *Urea* is always present in bile and usually in the same concentration as found in the blood and tissues. It is probable that its uniform distribution in all the body protoplasm and fluids is sufficient explanation for its presence in the bile.

Enzymes of various sorts are reported as present in the bile and these may or may not be associated with the activity of the specific hepatic or biliary epithelium.

Ethereal sulphates and *glucuronates* are found in bile but the reason for this is not understood. It is probably related to the recognized conjugating power of the liver epithelium (38).

Mucin is present in varying amounts in bile and is thought to be a secretion of the gall bladder epithelium. This substance is a nucleoprotein. The significance of this substance in the bile is not understood.

GALL BLADDER AND BILE DUCTS. The interrelation between the gall bladder, the bile ducts and the flow of bile has been the subject for many dissertations. It was recognized that bladder bile was much more concentrated than duct bile but it was debated whether this was due to absorption of water in the gall bladder or the addition of solids by the bladder epithelium. Rous and McMaster (45) have answered some of these questions by means of carefully controlled experiments. They show a remarkable power of bile concentration inherent in the gall bladder which in the dog can concentrate whole bile to ⅕ or ⅒ the original volume during a 24-hour period. Simple passage through the gall bladder may concentrate whole bile to ½ or ¼ its original volume. Rous and McMaster (46) further studied pure secretion of hepatic duct epithelium and found it to be water clear, of low specific gravity, containing traces of cholesterol.

Periodic discharge of bile from the gall bladder has been assumed but little studied. Recently experiments have been performed to throw light on this question by the use of dyes introduced into the gall bladder

(1). In dogs it was found that irritation of duodenal mucosa called out a flow of duct bile but *not gall bladder bile,* as has been assumed by many investigators using the duodenal tube and bile analysis. Stimulus of various nerves and even the wall of the gall bladder called forth no response and outpouring of bladder bile. Dyes introduced into the gall bladder were found after 1 to 3 days but not after 7 days. All this indicates a slow and very irregular filling and emptying of the gall bladder and our knowledge as to the controlling factors is obviously very fragmentary.

Bile secretion. The flow of bile in most animals with or without gall bladders is fairly continuous. There are periodic fluctuations in bile flow and bile production which may depend upon sphincter control of the bile papilla, stimulation of the duodenal epithelium, food factors, nerve stimuli, vascular changes, etc. When an investigator attempts to determine which single factor is concerned in any given bile flow reaction there is indeed need for caution and guarded conclusions.

The amount of bile produced varies with many factors—known and unknown. Much recorded data from human bile fistula cases is available (Stadelmann (51), Pfaff and Balch (39) and others). The volume in such cases may vary from 500 to 1000 cc. per 24 hours. A great amount of data on bile fistula dogs is available and some of these facts have been reviewed recently by Wisner and Whipple (66). Collections of bile for 24 hours in 6-hour samples show a number of interesting details. One is surprised to note little if any decrease in bile flow during night periods. These bile fistula dogs show no constant variation in bile pigment excretion during the different 6-hour periods. There are considerable variations in pigment output but the reasons for such variations are not clear. Bile acid production is much higher on a meat diet than on carbohydrate feeding, as noted above. On a meat diet one notes a slight falling off in bile acid secretion during some of the night periods but this is not so evident in the carbohydrate feeding experiments. For these dogs the volume output per 24 hours averages between 10 and 20 cc. per kilo body weight.

The *secretion pressure* of the bile is low in all animals. Values of 210 to 375 mm. bile are recorded for dogs and cats by Herring and Simpson (16) in acute bile duct obstruction. Similar values are reported by Mitchell and Stifel (34) in cats and dogs after chronic obstructions of 2 to 6 days. Similar observations in human patients have been recorded by Robitzchek and Turolt (42). The pressure in such cases was 210 to 270 mm. bile. Various drugs like atropin or pilocarpin have little or no effect upon this level of secretion pressure. Evidently there is a pressure level (commonly 210 to 300 mm. of bile) at which bile secretion and bile absorption are in equilibrium.

This obstruction pressure does not in any sense inhibit the production of bile and it is common knowledge that the bile constituents promptly appear in the blood and urine. This results in a clinical condition of icterus which does not concern us at this time. We may inquire as to the escape of the bile constituents from the bile passages in biliary obstruction. Mendel and Underhill (33) gave experimental evidence that dyes and chemicals injected into the bile passages escape by way of the blood stream and only incidentally by way of the lymphatics. Whipple and King (63) gave proof that the bile pigments in biliary obstruction escape promptly by means of the blood stream and appear in the urine. This reaction was not modified by a thoracic duct fistula and the bile pigments appeared more promptly in the urine than in the thoracic duct lymph after a biliary obstruction in the dog. We may conclude that during biliary obstruction the absorption of the bile constituents is effected mainly by the liver blood capillaries and only to a slight extent by the lymphatics.

BILE FLOW NECESSARY FOR LIFE? The question as to the necessity of bile to normal life and function may be answered in the affirmative. There may be doubts in the minds of physiologists and clinicians as to whether obstruction of bile or deviation of bile from the intestine to the exterior (fistula) will invariably give fatal results. We believe the evidence is becoming more convincing that bile is a necessary life factor. To be sure we observe human beings with long-standing obstruction who live months and "die of cancer." But it is not easy to state the cause of death in such cases nor simple to predict what would have been the story in the absence of the tumor complication.

Bile fistula animals appear to tolerate the exclusion of bile over considerable periods of time without serious impairment of health. Diet is an important factor in this equation. For example, a fistula dog will usually die within 2 months with acute intestinal disturbance if kept on an ordinary diet of kitchen scraps. A diet of milk, cooked potatoes, rice and bread will prolong life considerably and such dogs may live in good condition for 4 to 10 months and die with advanced bony abnormalities, as noted above. Abnormal pigment disturbances and true purpura with fatal hemorrhage may develop in such dogs. Addition of cooked liver to the above diet will usually improve the condition and prolong the period of health in these fistula dogs but they are apt to suffer at times from some of the conditions noted above.

Bile fistula dogs with tiny fistulous tracts connecting with the duodenum may live for long periods in perfect health and function. The amount of bile gaining entrance to the duodenum is very small and can scarcely exceed 10 or 20 per cent of the total flow which escapes through the external fistula. This shows how little bile may serve to change the entire clinical picture from abnormal to normal. The same amount of bile by mouth will not have any similar effect.

We feel safe in concluding, with certain minor reservations, that secretion of bile into the intestine is necessary for normal health and even for actual continuation of life beyond a few months' period.

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NON-PROTEIN NITROGEN OF BLOOD IN HEALTH AND DISEASE

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It has been known for nearly a hundred years that patients suffering from Bright's disease must be poisoned by their own waste products. These patients often passed little or no urine, and from them emanated the characteristic odor of decomposed urine, so that it did not require any unusual degree of scientific imagination to recognize the fact that the blood and tissues of these subjects must be loaded with the materials which normally find their way into the urine. For many decades the subject, nevertheless, did not progress very far beyond the concept conveyed in the term *uremia*. All attempts to show that the uremic coma is due to any specific urinary ingredient, such as urea, ammonia, potassium or the "extractives," were failures; nor did the investigations purporting to elucidate such specific causal connections particularly advance the available knowledge concerning the chemical composition of blood in Bright's disease, in other diseases, or in health. The early chemical proofs that normal blood contains urea were necessarily crude and would probably never have been accepted as proofs at all, if it had not been for the fact that the presence of urea was on *a priori* grounds so probable that no one seriously questioned it. Such tests as the identification of urea nitrate crystals (Schmidt, 1846), or the change in form of sodium chloride crystals produced by the presence of urea (1840–1855) were, of course, not capable of advancing the subject.

It is scarcely worth while to try to sift the intricate mixture of illusion, correct observations, and correct guesses which is represented in the early literature alike of the clinical investigators and the metabolism workers. Their reasoning power was necessarily much superior to their analytical skill or their meager laboratory facilities, and their analytical data could have been little more than mere ornaments attached to good, logical conclusions. The figures for some constituents might be fairly accurate; reasonably plausible figures for the urea content of different kinds of blood were published as early as 1850 to 1860.

The correctness of the concept that waste products must accumulate in the blood when the kidneys fail to perform their function in the formation of urine was established as early as 1821, when Prevost and Dumas showed that extirpation of the kidneys was followed by a gradual increase of the urea content of the blood—a finding repeatedly verified by other well-known investigators during several following decades.

Many recognized, of course, that little progress could be made on the basis of qualitative tests for urea or other urinary constituents in the blood, but it was one thing to see the need of suitable methods and quite another to find them. The earliest quantitative methods, borrowed from the field of urine analysis, such as weighing the isolated urea nitrate, or Liebig's mercuric oxide titration, or the laborious CO_2 method of Bunsen, were elusive tools in the hands of early nineteenth century clinicians. Kjeldahl's method for the determination of nitrogen did not come out until 1883, and before the discovery of that method there can scarcely be said to have existed the possibility of acquiring any comprehensive information concerning retention or the normal levels of the non-protein nitrogenous products in blood. The older Knop-Hüfner hypobromite method, in the form of innumerable modifications, was assiduously applied for many years after it had been abandoned by the chemists. This method is in fact still used in some places, notably in France. And, inaccurate as it is, it doubtless did good service, at least to the extent of keeping up interest in the problem of urea retention in nephritis. Widal's work is still largely based on urea determinations obtained by means of the hypobromite process.

While Kjeldahl's ingenious method supplied a sound basis for reliable determinations of the nitrogen, not only in blood, but in all kinds of biological material, its application to the study of the non-protein nitrogen in blood was neither particularly extensive nor particularly fruitful until Strauss (1) in 1902 made his well-known attempt to classify the various forms of nephritis partly on the basis of the amounts of non-protein nitrogen found in a large number of such patients. Strauss worked with blood serum. This comparatively early contribution of Strauss is now perhaps only of historical importance, as it has been replaced by more detailed and accurate classifications, such as those of Volhard (2), in which due attention is given to the rest-N of the blood. A detailed account of these important studies of various forms of nephritis does not come within the scope of this article because they are predominantly clinical.

The expression "non-protein nitrogen" has of late replaced the earlier term "uncoagulable nitrogen," while some writers have used the nondescript expression "rest-nitrogen." The terminology used is as yet of minor importance because it is not at present possible to describe all the nitrogenous products present in the filtrates used for analysis; and those products, moreover, differ to an appreciable extent according to the methods used for removing the colloidal protein materials. These differences in the total non-protein nitrogen of filtrates obtained by different methods of removing the albuminous materials are interesting, for all the methods in common use do remove the coagulable proteins and do leave in the filtrate the common waste products (or at least the urea), as well as the amino acids. The different values given by different processes for obtaining the filtrates indicate, therefore, that there are present in blood some products which are partly thrown down together with coagulable proteins and partly escape precipitation, and that the extent to which this is the case depends on the character of the method used for precipitating the proteins.

Abel (3) has obtained results indicating that these unknown products give certain protein reactions, and he suggests that they are peptones; while Folin and Berglund (4) have pointed out that since the products are more abundant in corpuscles than in plasma the products may belong to the histones. Neither of these interpretations covers the important fact that the unknown nitrogenous products in blood filtrates are greatly increased in bloods in which there is excessive retention of nitrogen, for in such bloods the undetermined nitrogen is even more abundant in the plasma than in the corpuscles. These facts are referred to here only to emphasize the point that it is not possible at present accurately to define the nitrogenous materials contained in the blood filtrates by means of which we study the "uncoagulable nitrogen" the "non-protein nitrogen" or the "rest nitrogen" of blood. The products contained in these filtrates can be classified in three groups:

A. The nitrogenous waste products.

B. Absorbed nitrogenous food materials.

C. Undetermined materials, including some undetermined waste products, some undetermined absorbed food products, and in addition some products of unknown origin.

It may be pointed out that the modern interest in the non-protein nitrogen of blood as an essential factor in the interpretation of normal protein metabolism was revived about the same time as the clinical importance of nitrogen retention began to take on added interest through

TABLE 1*

Sample analyses of protein-free blood filtrates obtained by means of tungstic acid

NUMBER	MGM. PER 100 CC. BLOOD					
	Total N	Urea N	Uric acid	Preformed creatinine	Total creatinine	Sugar
1	26	10	1.3	1.5	6.0	89
2	26	13	1.0	1.4	5.3	100
3	28	12	1.1	1.2	6.7	98
4	28	12	2.2	2.0	5.7	83
5	29	13	3.3	1.5	6.0	86
6	29	11	2.6	1.4	5.2	95
7	29	13	1.6	1.4	6.0	85
8	30	13	2.4	1.6	5.5	82
9	30	14	4.1	1.7	5.3	82
10	32	15	2.8	1.6	5.4	91
11	32	15	3.4	1.4	5.3	97
12	32	13	2.4	1.7	6.0	104
13	33	17	2.0	1.3	4.8	83
14	33	16	2.5	1.6	5.7	105
15	33	15	1.1	1.6	5.5	95
16	34	16	0.8	1.3	6.1	119
17	34	16	2.6	1.5	5.9	106
18	35	17	2.1	1.6	6.0	89
19	35	17	2.0	1.4	5.5	77
20	35	18	2.0	1.7	5.7	86
21	35	18	2.9	1.6	5.8	95
22	35	17	3.2	1.4	5.5	94
23	35	18	2.5	1.5	6.0	89
24	35	19	2.2	1.5	5.3	91
25	35	22	3.5	1.4	5.7	87
26	35	17	2.3	6.7	6.7	83
27	35	18	1.6	1.3	6.5	104
28	36	17	2.8	1.5	5.2	100
29	37	18	2.1	1.5	5.5	94
30	38	18	2.2	1.7	5.4	95
31	39	18	2.6	1.8	6.7	103
32	39	18	2.9	1.5	6.0	87
33	40	18	2.0	1.6	6.0	98
34	40	20	2.6	1.7	5.6	95
35	41	19	4.8	1.5	5.9	93
36	41	19	4.2	2.5	6.6	109
37	43	19	2.2	1.7	6.3	78
38	139	106	5.4	12.5	19.4	99
39	147	115	8.9	11.0	20.5	170
40	275	237	14.3	13.6	27.2	157

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* Folin and Wu: Journ. Biol. Chem., 1919, xxxviii, 109.

TABLE 2*

Normal minimum, maximum and average blood content of non-protein nitrogenous products (from 12 young men)

	WHOLE BLOOD PER 100 CC.				PLASMA PER 100 CC.				CORPUSCLES PER 100 CC.			
	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Undetermined rest N	Total non-protein N
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
After a night's fast												
Minimum.............	5.7	8.9	10.1	27.8	4.3	9.6	1.8	18.0	6.7	7.7	18.3	37.7
Maximum.............	7.8	15.2	17.5	39.4	6.2	17.3	11.5	30.0	10.7	13.2	33.8	55.0
Average...............	**6.4**	**11.5**	**13.7**	**32.1**	**5.3**	**12.4**	**6.7**	**24.7**	**8.2**	**10.3**	**24.7**	**43.6**
After carbohydrate intake												
Minimum.............	4.9	8.0	6.4	21.0	3.5	9.2	1.8	17.0	5.9	5.2	9.0	24.8

* Folin and Berglund: Journ. Biol. Chem., 1922, li, 415.

TABLE 3

Illustrating substantial uniformity in the retention of the several nitrogenous waste products (Not previously published)

CASE	WHOLE BLOOD PER 100 CC.						PLASMA PER 100 CC.						CORPUSCLES PER 100 CC.						CORPUSCLES
	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	Amino-acid N	Urea N	Creatinine	Uric acid	Undetermined rest N	Total non-protein N	
	mg.	*mg.*	*mg*	*mg.*	*mg*	*mg*	*mg.*	*mg.*	*mg*	*mg*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*vol. per cent*
1	5.0	13	1.8	3.7	19	39	4.3	13	1.8	**3.7**	20	**39**	5.9	13	1.8	3.7	**18**	**39**	**45**
2	6.4	49	2.0	4.5	7.4	65	5.8	47	2.3	**6.4**	18	**74**	7.2	52	1.5	1.6		**51**	**40**
3	4.5	49	3.0	4.7	22	78	3.5	52	3.0	**6.1**	18	**77**	7.4	41	3.0	0.9	**31**	**81**	**27**
4	5.8	60	7.7	4.8	37	107	5.4	71	8.1	**6.8**	21	**103**	7.2	26	6.5	0	**86**	**122**	**23**
5	7.6	91	7.2	6.4	41	144	6.2	109	8.1	**9.3**	23	**144**	11.8	38	4.4	0	**93**	**144**	**25**
6	8.4	174	12.9	13.6	75	267	7.5	214	14.5	**18.4**	52	**285**	9.7	113	10.5	6.1	**109**	**238**	**39**
7	7.3	193	16.0	12.0	48	258	7.3	234	19.2	**21.0**	51	**306**	7.3	138	11.7	0	**43**	**193**	**42**

the publications of Strauss. In 1902 Kutscher (5) had every reason to believe that protein is absorbed from the digestive tract in the form of amino acids, because he had previously proved that the tryptic digestion stops only with the complete transformation of protein into its component amino acids. Experimentally Kutscher nevertheless failed to find any evidence for an increase in the amino acid content of blood during active digestion, and he explained his negative results by accepting the prevalent doctrine of protein regeneration in the walls of the intestine. Cohnheim (6) found another strong reason for believing that the protein must reach the blood as amino acids when he discovered erepsin, but experimentally he also was unable to trace the absorbed nitrogenous materials into the blood. Abderhalden (7) likewise failed and explained his negative results as Kutscher had explained his. For a decade following the work of Kutscher and of Strauss more or less work was done; but very little was added to the findings reported by Strauss, and nothing to those of Kutscher. The underlying ideas survived, but experimentally the field remained unproductive.

In 1912 Folin and Denis (8) described colorimetric methods for the determination of the total non-protein nitrogen and for the urea and ammonia in blood, and a few months later Van Slyke (9) published a method for the determination of the amino acid nitrogen. In 1913 appeared the first colorimetric method for the determination of uric acid in blood: and the following year analogous methods were described for the determination of creatinine and creatine. Imperfect as the earlier forms of these micro methods of blood analyses were, their application revealed at once fundamentally important facts, which earlier investigators using improvised macro methods had sought but could not find.

In a series of papers entitled "Protein Metabolism from the Standpoint of Blood and Tissue Analysis" (1912–1914) Folin and Denis (10) traced the nitrogen of simple products, urea, amino acids, creatine and creatinine, through the walls of the intestine into the mesenteric and the portal blood, through the liver, into the general circulation, and into the muscular tissues. They also showed that the relatively high ammonia of the portal blood represented practically little else than the absorption of ammonia formed by putrefaction in the large intestine. The clearing up of this point was important in that it removed the chief experimental support in favor of the now discarded theory that the deamination process, that is to say, the hydrolytic removal of the amino groups from the amino acids, was localized in the intestinal mucosa. The experimental findings of Folin and Denis have on the whole been accepted as established facts, but some of their interpretations have not fared so well. In connection with similar experiments made by Van Slyke (11) attention was drawn to the fact that while the determinations of Folin and Denis disproved the current theory of localized deamination, they did not entirely disprove the older rival hypothesis of localized protein regeneration.

By means of his now so well-known method for the amino nitrogen determination, Van Slyke abundantly covered all that was missing in the evidence of Folin and Denis. Incidentally, Van Slyke showed that the blood always contains amino acids, and furnished the first dependable figures representing this fraction of the non-protein nitrogen. In one respect Van Slyke's findings, or rather interpretations, differed from those of Folin and Denis. The localized deamination process and urea formation which had figured so extensively in the literature, particularly American literature, during the preceding decade was again revived, but was transferred from the walls of the intestine to the liver, an organ which time after time has been represented as the chief seat of the urea formation. Van Slyke's interpretation is largely based on the interesting fact that the liver takes up an excessively large fraction of ingested or injected amino acids and then loses this charge in a relatively short time (3 to 4 hours). Van Slyke's interpretation of a localized deamination and urea formation remained without serious contradiction for a number of years.

Very recently, however, Folin and Berglund (12) have advanced another explanation of the temporary storage of amino acids in the liver, observed by Van Slyke, and have obtained a fresh series of analytical data on the amino acid absorption and the urea accumulation, which, in their opinion, indicates that the urea formation is a function of all mammalian tissues, and is not predominantly localized in the liver. Their evidence is based chiefly on data showing that the accumulation of urea in the general venous blood never precedes the accumulation of amino acids.

The question of the liver versus all the tissues in the body as the seat of the urea formation is of some importance clinically, quite apart from its place in the science of metabolism. "Liver function" tests in cirrhosis of the liver based on diminished urea formation or excessive amino acid excretion will stand in need of careful scrutiny and revised interpretation, if the liver is not normally the chief seat of the urea formation.

Ammonia. In its major aspects the character of the normal amino acid metabolism and its relation to the phenomenon of nitrogen equilibrium have been seemingly definitely settled by means of the modern methods for detailed analysis of the non-protein nitrogen. But new problems are constantly coming in to take the places of the old. According to the deamination process there should be no difficulty in getting a definite and true picture of the ammonia formation and its function to neutralize such acids as are formed and need to be neutralized in the tissues. If deamination and urea formation is not a localized process, there should be no occasion for any localization for the neutralization of acids by ammonia.

The results which have been obtained in determining the ammonia nitrogen fraction of the non-protein nitrogen have not been in good agreement with what one might expect from the quantities of ammonia which are found in the urine. The 24-hour ammonia nitrogen in the urine is usually between 0.3 and 0.6 gram in the case of normal persons, and in diabetic acidosis this ammonia may be increased as much as ten times, or even more. Corresponding to such variations in the ammonia excretion there ought to be unmistakable variations in the ammonia content of the blood filtrates, even though anything approaching a constant coefficient is not to be expected.

Because of the extraordinary difficulties involved in the determination of ammonia in blood most of the literature on the subject is taken up with discussion of the necessary analytical procedures, and after more than 20 years of such effort there is still no generally accepted method available. Obscure points, such as the ammonia content of every brand of potassium oxalate and the practical impossibility of removing that impurity by recrystallizations, the persistent presence of ammonia in every brand of ethyl and methyl alcohol, the formation of ammonia in the blood on standing, or on applying heat—all have contributed to the publication of ammonia values for blood which differ by several hundred per cent. The true values for blood remain, therefore, uncertain; and the uncertainty is increased by the fact that the blood of man yields substantially the same values as the blood of herbivorous or carnivorous animals, and substantially the same whether the blood comes from normal persons or from those eliminating excessive quantities of ammonia with the urine. The expected variations in the blood, corresponding to known variations in the elimination so far have not been found.

Nash and S. R. Benedict (13) have lately propounded an interesting explanation of the origin of the urinary ammonia which would account satisfactorily for all the observed facts. According to these authors the ammonia which finds its way into the urine is produced by the kidneys. The ammonia production might thus be pictured as another phase of the regulatory mechanism in that organ whereby the needed basic materials are reserved, partly by secretion of an acid urine and partly by this localized production of ammonia. Under these conditions the amounts of ammonia in the blood have, of course, practically nothing to do with the quantities excreted.

The explanation offered by Nash and Benedict is not merely a theory. It is supported by analytical data which show that the ammonia of the renal vein contains more ammonia than other blood, either venous or arterial. Opinions will necessarily differ as to the supporting value of the experimental data. The extra ammonia in the renal vein represents an over-production or a lack of perfect excretion of the ammonia, and is, of course, not at all an accurate index to the total ammonia formation in the kidneys—a point clearly recognized by the authors.

Nash and Benedict's paper gives an excellent review of the preceding literature. The one paper which has appeared later, Gad-Andersen's (14), describes another method for the determination of ammonia, but there is no reason to believe that this latest method is the best, especially as the results obtained are said to agree with those found by Henriques and Christiansen's method, which involves the use of four volumes of alcohol.

It is clear that for the present the question of the ammonia content of blood can have no bearing on any clinical problems.

Urea. Urea in blood as in urine is quantitatively by far the most important nitrogenous product. The normal variations of the urea nitrogen lie between 8 mgm. and 15 mgm. per 100 cc. of whole blood. The latter figure is really outside the normal, unless the subject is on a very high level of protein metabolism. In connection with upper normal values it should be pointed out that these values may persist for 2 or 3 days or longer after the protein consumption has been reduced. A low normal level is therefore not necessarily obtained, because the blood is taken before breakfast in the morning.

As a part of the total non-protein nitrogen of human blood the urea nitrogen varies under normal conditions between 35 and 55 per cent. The proportion falls most frequently between 40 and 50 per cent, but the variations are so large that it is not safe to assume, as is frequently done, that the urea nitrogen is just about one-half of the total non-protein nitrogen. In nephritic nitrogen retentions the increase usually involves a greater increase of the urea than of the total nitrogen, and the per cent of the latter represented by urea may rise up to 70 per cent.

Since the introduction of the urease methods for the estimation of urea (15), (16) this determination has become the most popular in chemical laboratories. The determination is unfortunately by no means so dependable as many seem to think. The enzyme employed is exceedingly sensitive, is occasionally more or less completely inactivated, and yields values that are too low. The total non-protein nitrogen determination represents, therefore, a more valuable and more dependable process for the study of nitrogen retention than does the urea determination. Both normally and in nitrogen retentions the urea is more abundant in the plasma than in the corpuscles. The distribution of the different nitrogen fractions of blood is illustrated by the table given on pp. 463 and 464. These figures were recently obtained by Berglund.

The enormous quantities of urea which can accumulate before the uremic patient finally dies indicate clearly that urea is not toxic, a fact also suggested by the extraordinary urea content found normally in the blood of the shark.

The simultaneous determination of urea in blood and in urine has received much attention in recent years as a means for determining the excretory efficiency of the kidneys. This line of investigation originated in France (17), and has been further elaborated by McLean (18). Opinions differ as to the value of such studies. The fundamental underlying assumption that the excretory power of the kidneys may be expressed in the form of a dependable constant is none too well established, however alluring it may appear to those who like to express metabolism processes in terms of mathematical formulas. The idea of the existence of such a constant certainly breaks down when it is extended so as to account for the rate of excretion of all waste products.

Uric acid. Of all the known definite constituents of the non-protein nitrogen there is none which has received so much attention and which is so interesting alike from the standpoint of normal and of abnormal metabolism as uric acid. It is difficult to appraise with any degree of certainty the work upon which the early findings of uric acid in blood are based. Garrod (19) cites figures for uric acid in human blood which, to be sure, seem not unreasonable. No one probably would now be able to confirm those figures by means of Garrod's method, yet Professor Lehmann in his well-known textbook (1851) states that he could for the most part confirm Garrod's observations—only (as he states) he first happened to do so by finding uric acid in the blood of carnivorous animals (dogs) (20). Yet 60 years after Garrod, Gudzent (21) made a notable contribution by merely showing that positive murexide reactions could be obtained on dialyzates from certain clinical bloods, but not from normal human blood or from the blood of animals. It is difficult to assume that Garrod in 1848 could tell more about the uric acid content of blood than Brugsch and Schittenhelm (22) could do a dozen years ago. These latter investigators devoted much time to the subject; but, like Gudzent, they had to be content with positive qualitative tests obtainable from certain kinds of human blood. Even after the extraordinarily delicate uric acid reagent of Folin and Denis had been introduced (1912) the uric acid content of blood could not always be determined with any high degree of certainty. Modern studies on the uric acid content of blood began with the introduction of that reagent.

A few remarks may be not out of place here concerning the interesting development of the colorimetric method for estimating uric acid in blood. The blood proteins from 20 cc. of blood were removed with boiling 0.01 N acetic acid. The filtrates were concentrated to a small volume and the uric acid was then precipitated by the best precipitant then known, the silver magnesium mixture—a uric acid precipitant first introduced by Salkowski in 1871. After removing the silver with hydrogen sulfide and boiling off the surplus H_2S the uric acid reagent was added, together with sodic carbonate, and a fine blue color suitable for quantitative comparisons was obtained.

This basic method has since been enormously simplified and improved. The most important single improvement was the introduction of potassium cyanide (23) by which the uric acid is set free and the disturbing silver is converted into complex cyanide compounds which have no disturbing effect. The next important step was the elimination of the tedious acetic acid precipitation, and accomplishing the precipitation of the uric acid by silver lactate without any preliminary concentration (24). The most remarkable step of all was taken by Benedict (25) a short time ago when he showed that it is not necessary to isolate the uric acid from the blood filtrates, and that by applying heat so much color is obtained that the blood filtrate from 0.5 cc. of blood is adequate for a determination. (Garrod used several hundred cc. ("2 pounds") of blood for each of his "determinations.") Benedict's last simplification is based on the use of a reagent which differs from the regular reagent in that a part of the phosphoric acid has been replaced by arsenic acid. Benedict believed that the secret of his success lay in this new reagent, but it has since been found (26) that the original uric acid reagent behaves in the same way and that the secret of the remarkable simplification introduced by Benedict lies in the use of appropriate amounts of sodium cyanide as the only alkali. Unless some now unknown flaw is discovered in these latest forms of the colorimetric determination of uric acid there is scarcely place for any further improvements, except perhaps in the matter of preparing more stable standard solutions of uric acid. This last requirement is probably met by Folin's finding that uric acid formaldehyde combinations liberate their uric acid quantitatively, when diluted with water.

The results of colorimetric determinations of uric acid in blood have on the whole substantiated the views which prevailed earlier both concerning normal purine metabolism and with regard to the accumulation of uric acid in blood in nephritis and in gout. The essential advance consists, therefore, up to date, mostly in the perfect certainty as to the correctness of these concepts and in the possibility of getting definite figures for all kinds of blood, including human blood in health and in disease. The fact learned from urine analysis that in all mammals, except the anthropoid ape and man, the chief end-product of the purine metabolism is allantoin, whereas in the anthropoid ape, in man, and in the birds, it is uric acid, is verified by the fact that in man and in birds the uric acid content of the blood is higher than in the blood of all mammals so far investigated. The uric acid content of bird's blood, curiously enough, is only about twice as high as the average normal found in man and is no higher than the values frequently found in the gouty.

The following uric acid figures for the bloods of animals were obtained by Folin and Denis (by the original colorimetric method) (27):

	RABBIT	SHEEP	PIG	HORSE	MONKEY	OX	CAT	CHICKEN
Mgm. uric acid per 100 cc. of blood	0.05	0.05	0.05	0.05	0.05	0.2	0.2	4.9

The figures 0.05 must be interpreted as meaning that no uric acid could be found, rather than that the figure cited is really correct.

In normal human blood the uric acid content is subject to relatively greater variations than that of any other known nitrogenous product. The lowest figure reported by Folin and Denis is 0.7 mgm., and the lowest found by Benedict out of 50 analyses is 0.8 mgm. The maximum normal figure for the uric acid may perhaps be given as 3 mgm. per 100 cc.

For typical cases of gout Myers (28) found uric acid values ranging from 6.8 to 9.5 mgm. without any accompanying increase of any other nitrogenous waste product. These figures average materially higher than the figures previously reported by Folin and Lyman (29) or by Folin and Denis. In twelve cases of gout unaccompanied by nephritis (normal non-protein nitrogen) these last two authors found uric acid values ranging from 3.3 to 5.2 mgm. The characteristic and extraordinary feature of pure gout is that in most cases only the uric acid is increased. Whether this peculiar fact is to be interpreted in terms of a highly selective activity and correspondingly selective deterioration of the kidneys, or whether it means that uric acid is more difficult to excrete than any other waste product, a possibility suggested by the high normal levels and by the excessive variations in the uric acid content of the blood of normal persons, is by no means clear; nor is it certain that either one of these two hypotheses can furnish an adequate explanation. In nephritis leading to true uremia there is a gradual accumulation of all the nitrogenous waste products, except ammonia, and in such bloods there is no constant relationship between the increase in the uric acid and in that of the total non-protein nitrogen. But, on the whole, one can say that the uric acid seldom, if ever, shows a greater percentage increase than does the total nitrogen; that the uric acid accumulation, on the contrary, is usually less pronounced. In other words, in cases of generally diminished kidney efficiency one finds no support for the idea that the uric acid retention represents any special difficulty in the process of excretion. The statement made above is true at least for advanced cases of nitrogen retention. Whether it is also or equally true for the early stages representing moderate nitrogen retention is not certain. Myers (30), Krauss (31) and others have drawn the conclusion that the retention and accumulation of the uric acid precedes and exceeds that of the non-protein nitrogen in the early stages of kidney insufficiency.

More observations on the problems are much needed, partly because the older colorimetric technic of uric acid determinations has been none too dependable—a situation not improved by the freedom with which little modifications have been introduced. Figures obtained by means of clinical colorimeters or by comparison with artificial standards can scarcely have much value in relation to such a problem. Values obtained on whole blood are also less convincing than those obtained on plasma.

There is one other interesting point which must be referred to in connection with the uric acid content of blood. Benedict (32) found some years ago that the uric acid content of ox blood which is normally very low (0.2 mgm.) increased several hundred per cent if the blood was allowed to stand (with preservatives) for several days. He also found that the uric acid content of blood filtrates from ox blood gave correspondingly increased uric acid figures if the filtrates were boiled with concentrated hydrochloric acid. Following these clues he was later able to isolate from ox blood filtrates a substance in pure crystalline condition which by hydrolysis is split into uric acid and a reducing sugar. Alice Rohdé Davis, working with Benedict on the problem, has obtained analyses indicating that the sugar is a pentose (ribose). This uric acid compound is located in the corpuscles of ox blood and is also found in the corpuscles of other animals, though in smaller amounts. If present at all in human blood the amount is so small that its isolation has not been accomplished with any degree of certainty. Morris (33) has lately obtained analytical data which, according to his interpretations, indicate that some form of combined uric acid may be present in some samples of human blood.

Creatinine and creatine. The investigation of these two substances as constituents of protein-free blood filtrates dates from the introduction of the colorimetric method for their determination (1914) (34). Earlier investigators had tried to determine creatine and creatinine as they had tried to search for every other product found in urine, and some ventured to report actual figures. Carl Voit (35), for example, found as high as 108 mgm. of creatine per 100 cc. of ox blood. All such early attempts were necessarily hopeless. Even the colorimetric method now used for the determination of creatinine and creatine has not yielded results the validity of which can not be questioned. Like the early findings for urea or for uric acid the modern creatinine and creatine values could not have been even tentatively accepted, if it had not been for the fact that creatinine and creatine were necessarily supposed to be present, since creatine is abundant in the tissues and since creatinine is one of the major nitrogenous waste products.

The earliest figures obtained by the colorimetric process were doubtless too high for the creatinine and much too high for the creatine. The figures for creatinine should be, and probably are, more nearly correct than those found for creatine, but like most figures obtained by a correct colorimetric reaction even the latest figures for blood creatinine must be interpreted as maximum figures, thus leaving open the possibility that they may later be shown to be too high. Up to the present time there is, however, no evidence available showing that creatinine figures obtained on the basis of really pure picric acid and the right amount of alkali are materially too high, at least when applied to plasma. Figures for the blood creatine are necessarily more uncertain, because of the heat which must be applied to the blood filtrate for the transformation of the creatine into creatinine. This heat was particularly disastrous when applied in the presence of picric acid, because some substance in the filtrate acted in acid solution on the picric acid so that it gave considerable color when the alkali was added. Folin and Wu thought that this effect might be due to hydrogen sulfide. (Hydrogen sulfide seems to have some reducing effect on picric acid when heated with it in the autoclave.)

Whether the plasma of normal adults contains any free creatine must, on the whole, be considered doubtful, since such individuals excrete no creatine (36), yet do excrete creatine when sufficient creatine is taken to show any demonstrable increase in the creatine content of the plasma. The true creatine present in blood is probably confined to the corpuscles, and the corpuscle creatine is probably held in these cells by the same forces which result in the maintenance of the creatine content of muscles. The significance of all this creatine has never been satisfactorily explained. Folin regards it as a (post-mortem) product set free when the cell protoplasm is killed (37). Until some other explanation of the sharp localization of creatine in the animal body has been found this explanation should be kept in mind as at least possibly correct.

The normal creatinine content of human blood may be given as 1.2 to 1.5 mgm. per 100 cc. of whole blood, according to results obtained by the method of Folin and Wu. The creatine content of such blood varies between 3.5 mgm. and 5 mgm. The creatinine content of blood is normally remarkably constant, as might have been expected in view of the fact that the endogenous production of creatinine is by far the largest source of the urinary creatinine. Because of these facts, V. C. Myers (38), with collaborators, has stressed the point that large retentions of creatinine represent the most valuable single index in advanced cases of nephritis in which there are nitrogen retentions. Further and more detailed investigations including particularly the concentrations in the plasma are urgently needed. Myers and Killian (39) found less creatinine in the plasma than in the whole blood in cases of excessive nitrogen retention. This finding is probably erroneous, and is presumably due to some flaw in the technic employed.

The question of the distribution of the various soluble nitrogenous products of blood filtrates between the plasma and the corpuscles is one which lately has received considerable attention partly because of the remarkable findings reported by Falta (40) and his co-workers. At present it seems measurably correct to say that if the different water content of plasma and corpuscles be disregarded, then only the amino acids, creatine, and the undetermined nitrogen are more abundant in the corpuscles than in the plasma; while the nitrogenous waste products—urea, creatinine, and uric acid—are more abundant in the plasma. In the case of uric acid the difference between the figures obtained from whole blood and from plasma is so large that practically the whole of the uric acid content of the blood falls on the plasma. If it were not for the greater practical difficulties it probably would be better for analytical purposes to substitute the plasma altogether for whole blood. Serum gives substantially the same values as plasma.

The determination of creatine, whether in whole blood or in plasma, is at present of importance only in connection with experiments which may serve to elucidate the obscure metabolic origin and significance of creatine and creatinine. Clinically such determinations can not at present be said to have any value. The creatinine determination, as already pointed out, is of considerable clinical importance.

Since the above summary was written there has appeared another paper on the creatinine and creatine of blood, by Behre and S. R. Benedict (41). In this paper the opposite side is taken on nearly every point involving the occurrence and significance of creatine and creatinine. The blood, including the plasma, is said to contain only creatine, and the creatinine figures previously reported are explained as due to the inadequacy of the available analytical technic. The creatinine content is given as less than 0.05 mgm. per 100 cc. of blood. The experimental work underlying these extraordinary conclusions is complicated. Behre and Benedict advocate the retention of the "creatinine" determination in the study of nephritis, although the chromogenic material now involved is entirely unknown. The determination should, however, not be made by the method of Folin and Wu but according to a process described by the authors.

In view of the many still active investigators who in the past have made contributions to the creatine-creatinine problem one can safely predict that the findings and conclusions of Behre and Benedict will not long remain without contradiction or verification.

Amino acids. The amino acid nitrogen content of blood has been already referred to. The problems of normal protein metabolism have been solved in a large measure, in so far as those problems are concerned with the fluctuations of the amino acid nitrogen of the blood. In connection with the investigation of clinical problems it should be pointed out that the determination of the amino nitrogen, by itself, has very limited application. The deamination process appears to be such a fundamental process that one cannot expect to find many pathological conditions in which the amino nitrogen of the blood filtrates will vary very much from the normal. As was stated in connection with the discussion of urea, the deamination is not affected by the accumulation of this end product. Even in the most advanced cases of uremic nephritis the amino acid content of the blood is by no means increased. In the course of a very large series of amino acid determinations on a great variety of clinical bloods Bock (42) has, to be sure, found unusually high values in nephritis when the non-protein nitrogen exceeds 100 mgm. The increases found are very variable. Bock used Van Slyke's method. Berglund, working with the colorimetric method, failed to find any increase of the amino acid nitrogen in several cases of nephritis, where the non-protein nitrogen ranged from about 70 mgm. to over 300 mgm. The normal values found by Bock ranged from 6.1 to 7.9 mgm.

That it is possible for deamination abnormalities to occur must be admitted. Cystinuria furnishes a beautiful example. For the present, however, such problems can be investigated only on the basis of urine analysis—unless one should by chance encounter a case of uremia superimposed on cystinuria.

The normal constant elimination of amino acids with the urine is interesting in view of the fact that the amino acids are not waste products. Their elimination indicates that there is no threshold, no force, by which they are quantitatively retained below a certain concentration in the blood. The level in the blood equivalent to from 5.7 to 7.8 of nitrogen per 100 cc. of whole blood, may be said to be normally very constant, but unmistakable increases occur after every substantial protein meal, and, corresponding to these small, but definite increases in the blood, there occur considerable increases in the hourly excretion. The reason why the daily loss of amino acids through the kidneys remains small is the same as the reason for the failure of the amino acids to accumulate in the blood—namely, the avidity with which they are taken up by the tissues. The tissues absorb the amino acids almost as extensively and as perfectly as they absorb the sugars, yet for the amino acids we assume no retentive mechanism analogous to the glycogen formation.

The statement made above that deamination abnormalities are rare and that one can scarcely expect to find such on the basis of amino acid determinations made on blood must not be construed as implying the complete absence of clinical cases in which the amino acid nitrogen of the blood is high. Extremely high amino nitrogen figures are already on record. In a well-studied case of yellow atrophy of the liver, Feigl and Luce (43) found almost a twenty-fold increase of the amino acid nitrogen (from 42 up to 115 mgm.). Stadie and Van Slyke (44) have also reported very high amino nitrogen values for an acute case of yellow atrophy (14 to 26 mgm.). Other similar cases have been reported. The high amino acid value found in such cases can scarcely be designated as representing metabolism disorders. The accumulation of amino acids is not due to a failure of deamination and urea formation, but rather to an excessive production of amino acids by virtue of a rapid autolysis of one large organ—the liver. The accumulation of amino acids in these cases is analogous to the accumulation of uric acid in leukemia.

Practically all of the literature on the amino acid content of blood is based on the gasometric method of Van Slyke. The new colorimetric method of Folin (45) appears to yield substantially the same sort of values, although as yet no parallel determinations have been made on blood filtrates.

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HARVARD UNIVERSITY
LABORATORY OF
GENERAL PHYSIOLOGY

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. II, NO. 4

Issued October, 1922

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the Act of March 8, 1879

Made in United States of America

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PHYSIOLOGICAL ABSTRACTS

Monthly, price 5s. net. Annual Subscription, post free, 40s.

This Journal is issued by the PHYSIOLOGICAL SOCIETY, acting in co-operation with numerous physiological organizations in Great Britain, America, and other countries.

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The Journal aims at issuing promptly abstracts of the papers published throughout the world, in physiological and allied sciences (including Plant Physiology).

A number is published at the beginning of each month.

Volume VI began with the April (1921) issue.

Subscriptions are only taken for April to March of the next year and are payable in advance.

Back numbers, excepting some which are out of print, may be obtained from the Publishers, or any Bookseller. An extra charge is made for some numbers the stock of which is nearly exhausted. Prices on application.

Binding Cases, price 2s. net, are supplied by the Publishers, who can also arrange for binding; prices on application.

PUBLISHED BY

H. K. LEWIS & CO., Ltd.

136 Gower Street LONDON, W. C. 1

Publishing Office, 28 Gower Place, London, W. C. 1.

SUBSCRIPTIONS MAY BE PAID THROUGH ANY BOOKSELLER

WAVERLY PRESS
The Williams & Wilkins Company
Baltimore, U. S. A.

PHYSIOLOGICAL REVIEWS

VOL. II OCTOBER, 1922 No. 4

THE PHARMACOLOGY OF THE AUTONOMIC SYSTEM

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A review of this subject, following so closely on the appearance of Langley's masterly little volume, *The Autonomic System,* requires unusual temerity. However, since most of the material had already been gathered and since the review had been planned from a somewhat different point of view, it seemed worth while to attempt to carry through the original enterprise; namely, a survey and orientation of the more recent developments that threatened or promised to disturb the narrow, rather rigid schema into which the pharmacology of the autonomic system had tended to "set."

The striking differences in the origin and distribution of the three great classes of efferent nerve-systems and their end organs—the somatic or voluntary system, the parasympathetic and the sympathetic—are reflected in an almost equally striking manner in their peripheral reactions to certain drugs; so much so that these could be definitely classed as sympathetic (for instance, epinephrin and ergotoxin); parasympathetic (for instance, pilocarpin and atropin); and strio-muscular (for instance, guanidin and curare). The correspondence between the drugs and innervations is so far-reaching that it cannot be accidental. There must be a fundamental, specific relation between the innervation and the drug-reactions; and if so, all poisons that act in a special manner on the peripheral system should belong, definitely and sharply, to one or the other of these groups; and we should thus be able to arrange all these drugs into a simple orderly, logical and generally satisfying system.

There is, however, another side of the picture: The correspondence is impressive, but not complete. On the one hand, some drugs affect, more or less, several of the systems; and on the other hand, the most typical drugs seem to act on the wrong system in some organs, such as the uterus and the sweat glands. These exceptions were naturally set aside as "atypical" until the main scheme could be better digested; but they have continued to accumulate in number and in impressiveness until it seems that they might submerge the whole schema, unless the schema could be sufficiently broadened to cover them. Very evidently, the divisions of the efferent systems are not separated as sharply as the schema would imply; at least, the chasm between them is not impassable. The facts may be reconciled by the assumption that the fundamental distinction goes beyond the nervous systems; that the reactions, whether to drugs or to nervous impulses, depend on the physical or chemical properties of the reacting cell; that these vary with conditions; that a certain complexion of these conditions is ordinarily associated with each of the nervous systems; but that these conditions may be shifted, naturally and artificially; and that then the reactions to nerve impulses and to drugs are also changed, separately or together.

In other words, it would appear that autonomic specificity is not an absolute, separate entity; but that it is conditioned on the ordinary, fundamental properties of muscle, of reactive tissue; in fact, of excitability in general. Conditions which affect these are also capable, potentially, of affecting autonomic specificity. This specificity is relatively fixed, because these properties are relatively fixed in a given type of tissue.

Generalizations of this sort hold out attractive prospects of satisfying the innate longing for unification; but they have little value unless they unify actual phenomena. Perhaps the chief significance of the more recent work on autonomic drugs is therefore the collection of data concerning the conditions that modify response. As yet, these data can furnish only hints, partly because they are incomplete in themselves, and partly because their interpretation must await clearer conceptions of the various properties of excitable tissues than we possess at present. There is reason to hope that each will throw light on the other; and it would not be surprising if the paradoxical effects of autonomic drugs would be important aids in unravelling the complexities of the nature of excitation.

Incomplete as are the present data, the space at the disposal of this review does not suffice to present them with any degree of detail. It can only sketch the subject in its broadest outlines, with a bit filled in here and there, by way of illustration.

I. Naturally transposed specificity. Under this heading we shall consider the instances in which drugs of pronouncedly specific autonomic character act on the wrong system, *under normal conditions.* Drugs with more ubiquitous actions—nicotin, barium, histamin, pituitrin, etc.—will only be considered incidentally. They are inherently eclectic rather than specific; and although the distinction may in the end prove to be of degree rather than of kind, they would as yet tend to obscure more than to clear the subject of perverted specificity.

It would also be desirable to disregard quantitative anomalies for the present. The various autonomic organs require quite different doses of their specific drugs, so that some may appear to be relatively refractory. The difference may be merely a matter of penetration, or it may have a deeper significance. In any case, changes of concentration may lead to reversal of the effects on the same system; or bring out effects on the complementary system. The quantitative anomalies therefore furnish a bridge between atypical actions induced by artificial conditions (dosage), and those that occur naturally with the usual dosage.

A special difficulty arises in deciding whether a reversed response (for instance, the secondary acceleration of the mammalian heart by pilocarpin) is due to a reversed action on the same system (conversion of vagus excitation to vagus paralysis); or to a transfer of the action to the complementary system (vagus excitation to accelerator excitation). It might appear that this could be easily decided by resort to nerve-stimulation or to antagonistic drugs; but the results of these methods are often as difficult to interpret as the original problem.

It is suggestive that perverted drug specificity is confined largely to organs, in which the innervation is itself unusual or confused. It is especially common when the tissue receives only one innervation; or when one innervation predominates very largely over the other; or where both systems produce the same response. For instance, in the sweat glands, the genito-urinary system; the frog's lungs; the blood vessels; and in the unpublished instance of the frog's small intestine, perverted specificity of drug is generally associated with weak contrast in the specificity of innervation.

We may conveniently arrange the instances under the innervations that are affected:

1. Sympathetic Innervations affected by Parasympathetic Drugs. a. The sweat glands: These are often quoted as the most striking instance of perverse autonomic reaction. Those of the cat's paw were described as innervated exclusively by the sympathetic, the electric stimulation of this causing secretion; but they fail to react to epinephrin, and on the contrary respond to the parasypmathetic poisons, to pilocarpin and atropin.

The work of Dieden and of Muto (1916), however throws doubt on the interpretation of the phenomena. They find that the sweat glands receive a parasympathetic as well as the sympathetic innervation; the relative importance varying in different animals. This would leave the possibility that the parasympathetic poisons act on a parasympathetic innervation. The drops of sweat that appear on stimulating the sympathetic could be due to contraction of the muscular coat of the glands, as suggested by Gaskell (p. 37); or the failure of epinephrin to produce secretion could be due to vasoconstriction.

The example of the sweat-glands is therefore not as decisive an instance of perverted specificity as is commonly supposed.

Incidentally, epinephrin has a normal augmentor effect on the sympathetically innervated skin-glands of the toad (Wastl, 1921).

b. The uterus: Although this has a double innervation, the parasympathetic appears to be practically negligible. Stimulation of the sympathetic (hypogastric) produces marked effects, which may be contractor or relaxor, according to the species of the animal. In cats, the response is inhibitory except during pregnancy, when it becomes contractor.

On pregnant cats, i.e., with contractor response of sympathetic stimulation, Cushny (1910) found that the parasympathetic pilocarpin produces contraction, as well as the sympathetic epinephrin. This has been abundantly confirmed, and seems to hold for all species and conditions in which hypogastric stimulation produces a contractor response.

The results as to the efficiency of the specific antagonists are conflicting. Cushny reported that the sympathetic ergotoxin antagonized the contractor effect of either drug; but that the parasympathetic atropin antagonized only the parasympathetic pilocarpin contraction, and not at all the response to epinephrin or to hypogastric stimulation. On the other hand, Dale and Laidlaw (1912) found ergotoxin ineffective against pilocarpin stimulation; and Gohara (1920) claims that atropin abolishes the contractor effect of epinephrin in rabbit's uterus.

There is also considerable uncertainty as to the transposition of inhibitory effects. In non-pregnant cats, in which hypogastric stimulation or epinephrin usually relax or inhibit the contractions, Cushny also noted inhibitory phenomena after pilocarpin injection; but they were much less characteristic than with epinephrin. Dale and Laidlaw did not observe relaxation when the excised non-pregnant uterus was subjected to pilocarpin, although it occurred sometimes during life. This they attributed partly to increased suprarenal output, partly to action on the sympathetic ganglia, and not a peripheral autonomic affect. Gunn and Gunn (1914), however, did occasionally observe inhibitory effects on the excised uterus of rats and guinea pigs.

These conflicting data as to the exchange of antagonisms, and as to the acquisition of inhibitory sympathetic characters by pilocarpin, do not invalidate the high importance of the undoubted fact that the contractor sympathetic innervation responds identically to sympathetic and parasympathetic drugs. It is evidently not necessary that a drug should completely lose its identity when it changes from one system to the other; and one need not expect that the sympathetic affinity of pilocarpin should reach the high perfection of epinephrin.

c. *Retractor penis:* This has a typical double innervation, with definite reciprocal response; the sympathetic being constrictor, the parasympathetic inhibitory. There would thus seem to be no good reason for transposition; but Edmunds (1920) finds that pilocarpin as well as epinephrin are contractor; that the pilocarpin contraction is antagonized by atropin; and that atropin does not prevent the inhibitory response of stimulation of the parasympathetic nerve.

There is thus apparently a complete transposition of the parasympathetic drugs to the sympathetic system; one might say that the contractor affinity of pilocarpin is in this case more powerful than its parasympathetic affinity. However, the transposition is not quite complete; for the pilocarpin is not antagonized by the sympathetically depressant ergotoxin.

d. *Urinary bladder:* This has also a double innervation; the parasympathetic being contractor, the sympathetic rather complex. The drug responses apparently are essentially true to type; the anomalies that have been described being probably merely quantitative differences of susceptibility.

Edmunds and Roth (1920) find that the differences which Elliott (1907) had found between the response to epinephrin and sympathetic stimulation disappears with appropriate doses. They themselves report that the atropin does not paralyze the response of the parasympathetic electric stimulation, although it antagonizes pilocarpin and physostigmin. This, however, is found in many organs; the nerve current seems more effective in overcoming the atropin block than are pilocarpin or physostigmin; or conversely, the dosage of atropin required to block stimulation of the parasympathetic nerves varies widely for different tissues (c.f. V. E. Henderson, 1922).

Other genito-urinary organs, so far as they have been studied, seem to have concordance of drug-specificity with innervation; whether this be double, as in the ureter; or predominantly sympathetic, as in the prostate (Macht, 1922).

e. Blood vessels: The evidence furnished by these is suggestive rather than crucial. They appear to have only a single innervation; at least, as judged by nerve stimulation, one nervous system or the other is so predominant that it appears exclusive. With most vessels this innervation is sympathetic and produces constriction. In the few situations where dilator effects have been definitely demonstrated, they are parasympathetic. However, drug reactions indicate that other vessels also have a mechanism for dilator stimulation through parasympathetic drugs, as shown most clearly for acetyl-cholin. This produces dilatation, which is antagonized by atropin (Hunt, 1918). Perhaps the parasympathetic "receptive substance" failed to establish connection with the nervous system because it is normally activated by the chemical products arising in the local metabolism.

The effects of pilocarpin are also dilator in most cases; but a few contractor reactions have been described, which would point to transposed stimulations of the sympathetic division. The data are cited by Langley (p. 37). Hildebrandt (1920) also reports that atropin antidotes epinephrin and sympathetic nerve stimulation in frog's vessels. It is not antagonistic to barium; but removes veratrin constriction (Kondo, 1919).

f. Heart: Pilocarpin inhibits the heart through vagus stimulation. This is followed by acceleration, expecially in mammals; in frogs, it removes muscarin standstill. This reversal is commonly attributed to direct depression of the vagus, which is found less responsive to stimulation. However, epinephrin also renders the heart irresponsive to the vagus (Langley, 1901; Kuroda and Kuno, 1916). This suggests that we may be dealing with a transposition of the pilocarpin stimulation to the sympathetic accelerators, and not with a direct transformation to parasympathetic paralysis.

g. Gastro-intestinal tract: One of the most striking instances of presumable transposition is furnished by the small intestine of the frog, whose behavior in saline baths has recently been studied at this laboratory by Dr. G. B. Roth (in press). It reacts in the usual manner to barium, by contraction; and to epinephrin by relaxation. The two drugs are mutually antagonistic, a matter of considerable interest for the localization of the barium action. Pilocarpin does not contract the intestine, as it does in mammals and turtles; or at most, there is a brief, faint and doubtful shortening; but to the contrary, it produces marked relaxation and antidotes the barium contraction, just like epinephrin. The pilocarpin relaxation is not antagonized by atropin, which is itself a depressant; although much weaker than pilocarpin. Physostigmin behaves like pilocarpin. The direction of the change does not appear to be influenced by the ion ratio or H-ion concentration of the bath.

In *other parts of the frog's intestine,* the pilocarpin and physostigmin response is in an intermediate stage: Fuehner (1918) found for the *stomach,* and Schuller (1921) for the *rectum,* that these drugs produce only a feeble contractor response; and in the rectum at least, pilocarpin prevents the contractor effect of the more fixedly parasympathetic arecolin.

A converse instance of the transposition of the parasympathetic poisons is furnished by the mammalian *ileo-colic sphincter.* Kuroda (1916) found that this is contracted not only by sympathetic stimulation and epinephrin, but also by parasympathetic stimulants; and that it is relaxed by atropin.

2. Parasympathetic Reactions provoked by Sympathetic Drugs. This transposition is less common; which conforms to the high degree of specificity of the sympathetic type of drug, epinephrin.

a. Lung-sacs of frogs and salamanders. The musculature corresponds to the bronchial muscle of mammals, but it has a different physiology and innervation (frog, Carlson and Luckhardt, 1920; salamanders, Luckhardt and Carlson, 1920). The muscle possesses a strong and continuous inherent tone, which is relaxed by parasympathetic (vagus) stimulation, and is slightly augmented by sympathetic stimulation. The response to epinephrin is transposed; i.e., it produces relaxation. Atropin presents another anomaly, for it does not prevent the inhibiting response to vagus stimulation. Pilocarpin which was tried only on salamanders, acts normally, i.e., relaxes.

In snakes and turtles, innervation and the response to drugs is as in mammals; contractor to parasympathetics; relaxor to epinephrin; except that the relaxor effect of epinephrin is still quite weak (Carlson and Luckhardt, 1920; Luckhardt and Carlson, 1921).

b. Blood vessels. As was discussed in a previous section, these generally respond by constriction to sympathetic stimulation; and by dilatation to parasympathetic stimulants. Although the sympathetic constrictor action of epinephrin is especially pronounced in the case of blood vessels, it produces dilatation in various situations; constantly so when lower concentrations of epinephrin are employed, or when the sympathetic excitability of the tissue has been lowered. It is not improbable that these are due to a parasympathetic transposition of epinephrin; this is strongly supported by the reaction of the pulmonary artery of frogs and turtles. In these animals, the constrictor innervation is not sympathetic, but parasympathetic (vagus; paralyzed by atropin); epinephrin, however, produces contraction, i.e., parasympathetic stimulation.

In other special vascular areas the drug-responses are still confusing. Considerable data for various drugs have been collected by the recent work of Amsler and Pick (1919) (frog-perfusion experiments, splanchnic areas and legs); Adler (1921) (ditto, lung and skin vessels); and Rothlin (1921) (rings of mammalian artery).

3. Autonomic Reactions provoked by Strio-Muscular Drugs. There seems to be a definite relation between the drug-affinities of the autonomic and strio-muscular innervations. Probably all of the drugs that act on the receptive mechanism of the striped muscles act also on the parasympathetic or sympathetic division, and most of the parasympathetic drugs act on striped muscle. The sympathetic division does not seem to affect muscular contraction and tone, but has been credited with influencing muscular metabolism.

The curare and nicotin group acts more especially on the autonomic ganglia, so that their effects on the terminal cells are not easily studied. *Guanidin* is perhaps more strictly terminal: The excised frog-heart (Rosenow, 1921) responds to weak concentrations by increased systole (sympathetic stimulation?); to higher concentrations by diastolic arrest (parasympathetic stimulation?). Bronchial muscle (Macht and Ting, 1922) is relaxed (sympathetic stimulation or parasympathetic depression?).

4. Strio-Muscular Reactions to Parasympathetic Drugs. The more powerful parasympathetic stimulants, physostigmin and acetyl cholin, stimulate the "receptive" mechanism of striped muscle in a manner strictly analogous to the characteristic strio-muscular stimulants, guanidin, nicotin and barium. The phenomena are the same for all, with mere quantitative differences that tend to disappear by varying the dosage: *a*, The lowest effective concentrations produce fibrillary twitchings; with increasing doses these pass to clonic contractions, tonus contractures, and finally into curare-like depression. *b*, With all, the action is strongest at the site of the end-plate; which might mean that the receptive mechanism is concentrated at this place; or merely that the permeability of the cell-wall at this place is more favorable to the penetration of the drugs or ions. *c*, All continue effective for some time after the division of the nerve trunks. Most and probably all become less and less effective when the nerve has completely degenerated, presumably because the receptive mechanism, and eventually the muscle fibers as a whole, deteriorate when they are no longer used. *d*, All are antagonized, though to a varying degree, by curare, atropin and especially l-scopolamin, and the local anesthetic group. Atropin is more effective against drugs than against nerve-stimulation; a difference that it frequently exhibits in strictly parasympathetic reactions, and which is again a merely quantitative question, for sufficiently large doses of atropin block the response of striped muscle to nerve stimulation (Haffner, 1918).

The data for these conclusions, but not necessarily the interpretations themselves are chiefly taken from papers of Frank and Katz (1921) (local anesthetics, nicotin, guanidin); E. Frank and Nothmann (1921) (physostigmin and scopolamin, on human); Frank and Stern (1921) (guanidin, degeneration, procain, atropin, barium); Fuehner (1920) (guanidin); Haffner (1918) (atropin); Langley (1913 and 1914) (curare and nicotin); Magnus (1908) (nicotin, curare, physostigmin); Meighan (1919) (guanidin); Riesser and Neuschloss (1921 and 1922) (acetyl cholin, atropin, curare, nicotin, local anesthetics); Schuller (1921) (local anesthetics and caffein); Schuller and Athmer (1921) (local anesthetics and veratrin).

5. Sympathetic Innervation of Striped Muscle. There is practically nothing to add to the review of this subject given by Langley on pages 69–80 of his book. Briefly, sympathetic fibers have been traced to the striped muscle fibers; especially by the degeneration method (Dusser de Barenne and co-workers, 1919). It was suggested on speculative grounds that this sympathetic innervation controls the sarcoplasm, and through it the tone of the muscle, in accordance with Bottazzi's sarcoplastic theory of tone. De Boer (1916) claimed to have demonstrated experimentally a loss of tone on division of the sympathetic supply. Dusser de Barenne (1916)and S. Cobb (1918) showed that this has essentially no effect on the tone. The sympathetic innervation was then credited with the regulation of the metabolism of the muscle, but without direct evidence. Langley points out the difficulty of distinguishing a sympathetic innervation of the muscles from a sympathetic innervation of the blood vessels in the muscles, and does not consider the evidence quite conclusive; but inclines to the positive side; i.e., that sympathetic nerves make direct connections with the muscle cells.

The behavior of striped muscle toward epinephrin supports the view that the sympathetic innervation does not act on muscular contraction or tone; for epinephrin does not affect these in excised muscle. On the other hand, epinephrin increases the carbon dioxid production, even in excised muscle (Martin and Armitstead, 1922), and also creatin formation (Riesser, 1916). This indicates that the sympathetic innervation may be concerned with possible functions of muscle in general metabolism; perhaps in heat-regulation.

II. TRANSPOSITION OF SPECIFIC ACTIONS BY ARTIFICIAL CONDITIONS. We have seen that normal specificity is not absolute; that the reactions of the parasympathetic and sympathetic drugs are sometimes transposed, sometimes intermingled. It is therefore evident that innervation, although the most important condition, is not the *only* condition concerned in the specificity of the autonomic drugs. These conditions could be reduced in principle to two, sensibility and dosage. Each of these in turn may be changed by a large variety of factors, of different relative importance.

Sensibility and dosage determine, in final analysis, the degree of normal action; reversion of normal action from stimulation to depression or conversely; and transposition to the other system. These are all reducible to the same principles. It will facilitate the exposition if we employ the following terms as indicated:

Reversal of function: Response to the organ in opposite directions (for instance, change of contraction to relaxation). This may be the result either of transposition or of transformation of action:

Transposition of action: From the normal to another system (for instance, an epinephrin effect on the parasympathetic innervation).

Transformation of action: The action being on the normal system, but in the opposite direction (for instance, a nicotin stimulation changing to a paralysis).

Sensitization and desensitization will be used for increased, respectively diminished, response of either normal or the transformed or the transposed actions.

Further discussion will be more profitable in connection with actual examples:

1. Action transposed by Intensity of Electric Stimulation: The claw of the crayfish is moved by a stronger adductor and a weaker abductor muscle. Either muscle can be placed in tone by the removal of the other. When the muscle, with its contained nerves, is then stimulated, the response varies according to the strength of the stimulus and the muscle:

Weak stimulation relaxes the adductor and contracts the abductor.

Strong stimulation contracts the adductor and relaxes the abductor.

Each muscle has a separate contractor and inhibitor innervation (the literature is cited by Gaskell, p. 74–76). It is clear, therefore, that merely increasing the strength of the stimulus transposes it from the relaxor to the contractor innervation in the case of the adductor; and from the contractor to the relaxor innervation for the abductor.

The central nervous system furnishes numerous instances of transposition of action according to the strength of the afferent impulses, which may be more or less analogous; but it is advisable to confine ourselves for the present to peripheral reactions.

2. Paradoxic Cardiac Effects of Autonomic Poisons. In the excised frog heart, fed by the Straub cannula, the simultaneous action of sympathetic and parasympathetic stimulants does not result in neutralization of their effects, as might be expected; but in transposition of the stimulant action of one of the poisons to the other system. The Vienna school (Pick, 1920) therefore assumes that both groups of drugs may stimulate either of the autonomic systems, i.e., that they are really "amphotropic." They act, however, with different intensity; so that the stimulation of the complementary innervation becomes manifest only when its excitability is abnormally raised, or if the excitability of the usual innervation is abnormally depressed.

a. When under the moderate influence of parasympathetic stimulants (acetyl-cholin, etc.), the heart responds to epinephrin, not by the usual sympathetic augmentory response, but by increase of the parasympathetic diastolic, inhibitory action. This inhibitory effect is removed by atropin, so that it appears due to parasympathetic stimulation (Kolm and Pick, 1920). Analogous transposition is shown by the blood vessels and intestines.

Pick believes that the production by epinephrin of cardiac fibrillation and dilatation in early chloroform anesthesia is due to an analogous transposition, assuming that the vagus is sensitized by small doses of chloroform.

b. Nicotin and ergotamin also produce a similar transposition of epinephrin to inhibitory action (Amsler, 1920). Amsler assumes that the ergotamin paralysis of the sympathetic reciprocally increases the sensibility of the parasympathetic, and thus transposes the epinephrin action. This would also furnish the explanation of epinephrin "reversal" in other organs.

He also explains the nicotin-epinephrin phenomenon by paralysis of the sympathetic; but Hett (1920) finds that nicotin acts on the heart as a parasympathetic stimulant; so that the transposition may be more strictly parallel to the cholin-epinephrin phenomenon.

c. Physostigmin also sensitizes the heart to the diastolic action of strophanthin to such an extent, that even maximal doses produce diastolic arrest instead of the usual systolic standstill (Froehlich and Pick, 1920). Since systolic standstill seems to be connected with sympathetic stimulation, the strophanthin diastole may be conceived as a transposition.

d. Conversely, sensitization of the sympathetic by epinephrin, or by excess of calcium transposes the parasympathetic poisons (muscarin, acetylcholin, etc.) to sympathetic stimulation; so that they produce systolic contracture instead of diastolic arrest. This sympathetic action of physostigmin is prevented by ergotoxin, and not by atropin.

e. Abolition of ventricular vagus response by excessive doses of vagostimulants. The mechanism of this phenomenon is not clear; but it may receive mention in this place. Large doses of apparently all vagus stimulants (aconite, muscarin, physostigmin, digitalis, acetylcholin, pituitary) render the ventricles irresponsive to vagus stimulation. The sinus and auricles still react by diastolic standstill, so that the interference appears to be with the conduction of the inhibitory impulses (Froehlich and Pick). It is therefore possible that the action does not consist in reversal to the vagus effect, but that it may involve some other mechanism of the heart.

3. The Influence of Electrolytes on Cardiac Response. The ions of serum and saline solutions influence profoundly all the functions of the heart, and among them their response to autonomic stimulation by drugs. These ion effects are very complicated, confusing and difficult to analyze; partly because they depend on the ratio, for instance of Ca:K, as well as on the absolute quantity of each; and partly because different levels of the heart react quite differently, and sometimes in opposite directiosn. The practical importance of this latter fact is not yet sufficiently widely realized; for it determines the gradient of irritability, of tone and of rhythm production; so that the "pace-maker" may be shifted at will by changes in the ion ratio (Sakai, 1914; Daly and Clark, 1921; Kolm and Pick, 1920). Presumably this applies also to peristaltic progression in general; for instance in the intestines. However, changes in ion-ratio sufficient to influence autonomic reactions are not readily produced in intact animals (Schafer, 1915).

A detailed discussion to the ion-effects would be beyond the scope of this review. The more recent data can be found in the papers of Daly and Clark, Kolm and Pick and Carter and Andrus (1921).

a. In general, changes in the potassium and calcium content act in opposite directions:

Increase of the $\frac{K}{Ca}$ *ratio* (i.e., *Calcium deficiency or potassium excess*) produces the typical phenomena of parasympathetic (vagus) stimulation, culminating in diastolic standstill (e.g., Burridge, 1912).

Increase of the $\frac{Ca}{K}$ *ratio* (i.e., *Calcium excess or potassium deficiency*) produces the phenomena of sympathetic (accelerator) stimulation, culminating in systolic standstill (Kolm and Pick, 1920).

In brief then, *calcium* seems to sensitize the sympathetic or desensitize the parasympathetic, and *potassium* seems to sensitize the parasympathetic or to desensitize the sympathetic mechanism.

(These simplified statements hold only for solutions that contain Ca, K and Na ions. The phenomena are modified if one of the constituents is entirely absent; for instance, entire absence of Ca abolishes the response to vagus stimulation (O. Loewi, 1917).)

b. When the ion-changes are combined with the other autonomic stimulations or drugs, they reinforce or transpose the effects, in the same direction as if the ordinary specific autonomic poisons were combined:

Increase of the $\frac{K}{Ca}$ *ratio* (*Calcium deficiency or potassium excess*), corresponds to sensitization of the parasympathetic (vagus) and enhances the response of the parasympathetic stimulants (muscarin, cholin, etc.) and transposes the action of sympathetic stimulants, so that the epinephrin now produces a diastolic effect (Burridge, 1912), which is antagonized by atropin, but not by ergotoxin (Kolm and Pick, 1920).

Increase of the $\frac{Ca}{K}$ *ratio* (*Calcium excess or potassium deficiency*) corresponds to sensitization of the sympathetic (accelerator) and therefore enhances the response to epinephrin, and transposes the action of parasympathetic stimulants, so that acetyl-cholin produces systolic instead of diastolic standstill. The action is antagonized by ergotoxin, not by atropin (Kolm and Pick).

Increase of the $\frac{Ca}{K}$ ratio also sensitizes to systolic standstilll by *strophanthin*, whilst increase of $\frac{K}{Ca}$ causes strophanthin to produce diastolic standstill (Loewi, 1915).

c. Sensitization of the sympathetic or desensitization of the parasympathetic is also produced by non-electrolytes; by lecithin and soaps; by serum; by increase of alkalinity; and by seasonal changes:

The presence of *non-electrolytes* (urea, sugar) renders the heart more resistant to calcium deficiency; which Loewi (1921) interprets as sensitization to the smaller quantities of calcium that remain. The non-electrolytes also sensitize to strophanthin; they may therefore be classed with the sensitizers of sympathetic action.

Treatment of the heart with *lecithin* and *soap* renders it more resistant to cholin and pilocarpin, i.e., diminishes parasympathetic stimulation and would therefore sensitize to sympathetic stimulation (Loewi, 1921).

Blood serum antagonizes parasympathetic stimulation (muscarin), and increases the parasympathetic depression of atropin. This is due partly to its calcium, partly to the ether-soluble extractives (Kirste, 1921).

Increase of *alkalinity*, within physiological limits (to H_p–7.8) increases the tonus (Andrus, 1919); i.e., acts in the sympathetic direction.

The Aestival vagus insusceptibility of frogs is not due to changes in the nerves, but to desensitization; for the inhibitory response can be restored by drugs that stimulate the vagus or that depress the sympathetic (Cori, 1921).

Radioactive substances: These seem to act in the direction of parasympathetic sensitization; for they seem to have the effect of radio-equivalent quantities of potassium (Zwaardemaker, 1918). Zwaardemaker suggests that the radio-energy liberates potassium (and presumably other ions) from their combinations in the protoplasm.

4. Paradoxic Vascular Reactions, and Influence of Electrolytes: The blood vessels exhibit the same transposition of specific autonomic drugs by sensitization of the opposing system or desensitization of the normal system, as was described under the heart; either through specific autonomic drugs, or through changes of the ions in the salt mixture, or through other means.

a. Dilator action of epinephrin. The ordinary constrictor action of epinephrin is identified with sympathetic stimulation. Under certain conditions this is replaced by a dilator reaction, which is presumably dependent on parasympathetic affinities; this becomes manifest especially whenever the sensitivity of the sympathetic innervation is lowered; as by degenerative nerve-section (Pearce, 1913; Engelock, 1915); by prolonged sojourn of the tissue outside of the body (Sollmann, 1905); or by very prolonged perfusion with epinephrin itself (Ogawa, 1912; Bauer and Froehlich, 1918); and by autonomic drugs and ions that depress sympathetic or increase parasympathetic reactivity.

Very small doses of epinephrin are also credited with a dilator action; it is not known whether this depends on the same mechanism.

The normal dilator response of certain vessels (studied by Rothlin, 1921) may be due to the normal preponderance of parasympathetic irritability.

b. Sensitization by specific autonomic drugs. The transpositions through the presence of other specific autonomic poisons are identical with those described in section II, no. 2, for the heart (Kolm and Pick, 1920).

Spleen extract also sensitizes to the pressor (sympathetic) response of epinephrin; and antagonizes the depressor (parasympathetic?) response (Collip, 1920).

c. Relation to electrolytes. Calcium appears to act in the opposite direction in vessels, and in the heart. Potassium and hydrogen and hydroxyl ions act on the whole in the same direction in both; (Schmidt, 1921, frog perfusion):

Calcium excess acts as a parasympathetic stimulant to the vessels, and as a sympathetic stimulant in the heart. The vessels are dilated, and if the constrictor response to sympathetic nerve stimulation and to epinephrin is diminished, they may even respond by dilatation.

Calcium deficiency acts as a sympathetic stimulant on the vessels, and as a parasympathetic stimulant on the heart. The vessels are constricted, and the constrictor response to epinephrin is enhanced.

Potassium excess acts as sympathetic stimulant to the vessels; as a parasympathetic stiumlant on the heart. The vessels are constricted. However, the effect on epinephrin is abnormal; the constrictor effect is diminished, or may even be replaced by dilatation.

Potassium deficiency also acts as a sympathetic stimulant, to the vessels, as well as to the heart. The vessels are constricted and react more effectively to the constrictor action of epinephrin.

Alkalinity increase: Moderate increase acts in the direction of sympathetic stimulation for the vessels, as for the heart; i.e., perfused vessels constrict. This is not antagonized by ergotoxin. Epinephrin is effective (Heymann, 1921). Moderate alkalinity also promotes the contractions and tone of excised arteries (Opitz, 1920).

Large excess of alkalinity relaxes (Heymann).

Diminished alkalinity and increased acidity: Diminished alkalinity also constricts the perfused vessels (Pearce, 1913). The mechanism is not clear; the constriction is prevented by ergotoxin, so that it might be considered as sympathetic; but the constrictor response to epinephrin and to nerve stimulation is abolished or replaced by a dilator response (Heymann; Schmidt).

A larger excess of acid dilates the vessels; the dilator concentration coinciding with the H ion concentration that stimulates the respiratory center (Adler, 1921).

Transposition of action by sensitization and desensitization applies also to the other organs on which it has been studied; for instance, the intestines (Kolm and Pick). It is not necessary to occupy space in their discussion further than to point out that many details remain to be elaborated.

III. PERIPHERAL POINT OF ATTACK OF THE AUTONOMIC DRUGS. The energy and ingenuity that have been spent on attempts to localize the site of the action of autonomic drugs has not yet borne full fruition. Indeed, the subject has been full of disappointments—or of interest, according to the point of view. Again and again, conclusions that appeared securely established had to be revised. Methods of experimentation and deduction that appeared conclusive were found, on further examination, to be inadequate and misleading. Differences that appeared fundamental turned out to be only quantitative, subject to exceptions, dependent on conditions. Magnus in 1908 destroyed confidence in localization by antagonists, but considered anatomic methods of isolation as conclusive; it has since been shown that these may also lead to false conclusions, by being incomplete, or by injury to other tissues, or by indirect reactions through tone, reciprocal innervation, etc. Even degeneration of nerve-endings has led to deceptive conclusions.

This experience may seem discouraging as to theories; but it is inspiring to the search for facts: for the destruction of the theories has been brought about by, and led to, the accumulation of data. The theories have therefore served their normal useful fertilizing function.

To proceed with the consideration of the points of attack: Autonomic drugs could conceivably stimulate or depress either of the two systems by acting on four principal mechanisms:

1, On the ganglionic elements; *2*, on the nerve-endings; *3*, on the receptive mechanism or excitability of the terminal cell; and *4*, on the responsive mechanism, the contractility or secretory activity.

1. The Ganglionic Elements. The ganglia of invertebrates are susceptible to the whole series of autonomic drugs; for instance, the ganglion-chains of the marine worm, sipunculus (Magnus, 1903); or the cardiac ganglia of the king-crab (Carlson, 1906). With vertebrates, the autonomic ganglia react definitely to the nicotin group, as shown by the fundamental work of Langley; but wherever they can be separated with sufficient sharpness, they do not seem to be very susceptible to the other sympathetic and parasympathetic poisons. It is more difficult to confirm this conclusion absolutely in situations where they are inseparably intermingled with the terminal elements, as in the heart and in the intestines. The analogy of the limulus heart lent color to the suggestion that the ganglion cells might be the seat of the rhythmic activity and of the actions of drugs upon it. The analogy, however, is not binding, and the "neurogenic theory" has almost hopelessly lost ground for the vertebrate heart (Eyster and Meek, 1921). As concerns the autonomic drugs these act on the ganglion-free apex essentially as on the whole heart (S. Loewe, 1918; Sasaki, 1921).

The evidence for the importance of the intestinal ganglion plexus to the autonomic reactions rested essentially on the experiments of Magnus (1904) that seemed to show that the rhythmic contractions of the intestines, and the effect of drugs upon them, depended on the integrity of the Auerbach plexus. Later work, however, showed that the absence of rhythm was due merely to traumatic injury; Gunn and Underhill (1922), showed that plexus-free preparations are rhythmic; and that the rhythm can be evoked for days after the excision, whilst the ganglion cells are assumed to die quite promptly. This has been abundantly confirmed (for instance, Alvarez and Mahoney, 1922); and holds true also for the peristaltic movements of the excised intestine, as shown by the method of Trendelenburg (1917). The results of these indicate that origin and direction of the peristaltic wave depend on a gradient of tonus and excitability, inherent in the muscle; which in turn depends presumably on adjustment to the optimum ion ration. The effects of drugs in revived intestine also agree essentially with those in intestine in which the ganglia are alive.

Carlson and his co-workers find another argument for the independent activity of the Auerbach ganglia in the capricious response of the cardial region of the stomach to nerve stimulation: either vagus or sympathetic may produce either contraction or inhibition (Carlson, Boyd and Pearcy, 1922). From this they argue that these nerves do not act directly on the muscle, but that they are largely afferent nerves to peripheral reflex center, i.e., to the ganglion plexus. The deduction does not appear conclusive. It is conceivable that each nerve might carry both augmentor and inhibitory fibers directly to the muscles (as Tashiro (1920) assumes for the intestinal sympathetic), or end result of the same stimulation on a given muscle fiber may be modified by the state, especially the tone, of that fiber. On the whole the facts seem in harmony with Gaskell's view that the ganglion cells of the autonomic system are connector elements, analogous to the pyramidal cells of the motor system, but transplanted to the periphery. However, the final conclusion must be left to the future.

2. Nerve-Fibrils and Endings. Nerve-fibers, where they can be reached separately, are practically unaffected by most of the specific autonomic poisons. It is therefore improbable that the finer terminal fibrils, which seem to be mere continuations of the axis-cylinders, should be the site of the specific actions. They differ from the larger fibers in permeability, it is true; but this would cause only quantitative differences, as is shown by the local anesthetics.

Direct evidence against the nerve fibrils is furnished by the persistence of the responses to autonomic drugs in excised organs after the excitability of the nerve fibers has been lost.

The results after degeneration of the nerve fibrils and endings, following division of the nerve trunk, speak in the same direction. The effects of a number of autonomic drugs, notably epinephrin, can still be evoked after complete degeneration of the nerve. This localizes their action definitely beyond the termini that are in nutritive connection with the nerve.

The interpretation of the converse phenomenon, disappearance of the drug response as the result of degeneration, is not so decisive. The effects of pilocarpin, for instance, can be evoked like those of epinephrin, after degeneration, i.e., when stimulation of the nerve trunk is ineffective. After a time however (6 weeks for sweat, Anderson and Langley, 1904) the response becomes less efficient although the muscle still responds to electric stimulation. This probably means that in the end the muscle cell also deteriorates, "atrophies," when it is not functionally used; and that this deterioration abolishes its excitability to pilocarpin before it abolishes the contractor response to direct electric stimulation.

The response to physostigmin disappears in general earlier than the response to pilocarpin: For the eye, within a few days (Anderson, 1905); but for the fibrillary contraction of striped muscle only between the 18th and 27th day (Magnus, 1908). The graduation suggests that the same mechanism is involved as with pilocarpin, i.e., gradual degeneration of the muscle.

It is rather unexpected that the excitability to physostigmin should disappear before the excitability to pilocarpin, which is a less powerful stimulant for innervated muscle, and the less powerful antagonist of atropin; but that does not prove that physostigmin acts on the nerve endings. It may be, for instance, that the physostigmin requires a special configuration of the receptive mechanism that degenerates relatively more rapidly; or it may be that pilocarpin actually excites the receptive mechanism, whilst physostigmin acts more as a sensitizer to other excitations, which would be excluded as degeneration proceeds. In favor of this explanation is the fact that the smaller doses of physostigmin only lower the threshold of muscle (normal) to nerve stimulation; that somewhat larger doses cause fibrillary twitching only after electric stimulation, and that spontaneous twitchings require a still higher dosage (Langley and Kato, 1915).

It may therefore be concluded that there is no conclusive evidence that any of the specific autonomic poisons act on the "nerve-endings" that degenerate after section of the nerves; and that the evidence of excised organs is distinctly against this mechanism.

3. The Receptive Mechanism. The fact that epinephrin contracts some smooth muscles, and relaxes others of apparently identical structure and which respond alike to direct electric stimulation, implies that it does not act directly on the contractile muscle substance. The striking correspondence with the response to sympathetic nerve stimulation implies that it acts on a nervous mechanism, but the persistence of the response after nerve-degeneration proves that it does not act upon the nerves proper. It therefore became necessary to assume the existence of some intermediate mechanism, related to the innervation, but not trophically dependent upon it, on which the epinephrin and perhaps the nerve current must act. Epinephrin does not stand alone in this respect; probably all specific autonomic poisons act through the mediation of a recipient mechanism; and even some poisons that were formerly believed to affect the contractile substance directly.

The intermediate mechanism may be conceived in several ways: *a*, as a morphologic structure; *b*, as a chemical receptive substance or side-chain; *c*, as a physical system. The available facts do not permit a decision between these; indeed, the theories themselves lack so much in detail that a decisive attitude toward them would not be justified. Consequently, there is an advantage in employing a non-committal term, such as "*receptive mechanism*" for the entire concept; and to restrict the more specific terms, "myo-neural junction," "receptive substance," and "synapse," to the special theories that they represent.

These theories should be examined in the light of the characteristic phenomena of the autonomic system: the fact that the autonomic cells are usually innervated by two systems of nerves; that either of these may be augmentory or inhibitory in different situations; that autonomic drugs act specifically on one or the other of these systems, in one direction or the other; and that these actions may be transposed or transformed. Any theory of the receptive mechanism must be compatible with these phenomena, and if possible seek to explain them. This task may not prove as formidable as it sounds.

a. Morphologic structure intermediate between nerve and muscle; the "myo-neural junction" of Brodie. This assumes that the nerves are continued into the muscle cell, and there become modified in such a way that they do not degenerate on section of the nerve trunk. This attempts only to account for the persistence of the autonomic drug-response after nerve-degeneration; it leaves untouched all the other phenomena we have indicated and is difficult to reconcile with some of these, for instance, the transposition of action. The direct evidence for it is slender and dubious; and even if non-degenerating endings exist, this need not mean that they are the site of the action of the drugs; and if they are, this could be made a part of either of the other theories.

The direct evidence for the intermediate structure consists essentially of the "endplates" of striped muscle: these are definite structures, which might possess modified nervous functions; and the nerve fibrils themselves apparently lie within the sarcolemma, in close apposition to the nuclei, which might modify their nutrition. However, Langley (p. 49) cites Boeke that the intracellular network does degenerate after nerve section, which speaks against rather than for the myo-neural theory; and the nucleo-sarcoplastic part of the sole-plate cannot be invoked, since nothing of the sort is present in the cells of the autonomic system proper.

b. Chemical receptive substance. Langley assumes that the autonomic drugs combine chemically with constituents of the cell—the receptive substances, or rather perhaps labile side-chain receptors of the molecule of the general cell substance. He considers that two classes of these receptors are necessary, one giving rise to contraction, the other to inhibition; the response to sympathetic or parasympathetic stimulation depending on the relative amount of contractor and inhibitor receptive substance "connected with them in the cells."

The two receptive substances could be made to account for the response to nerve impulses, but it seems difficult to apply them to their original object, the response to drugs. It is not clear, for instance, on what epinephrin is supposed to act. If simply on the receptor substance, then why should it have any relation to the sympathetic nerve supply? If it acts on the receptive substance only if this is connected with the sympathetic nerve supply, then the receptor theory loses its chemical character and becomes essentially a theory of specific difference in the nerve itself—a supposition that Langley himself rejects. The only alternative seems to be to assume four receptive substances—sympathetic contractor, sympathetic inhibitor, parasympathetic inhibitor, and parasympathetic contractor; and by buttressing this with a sufficient number of other subsidiary assumptions, explanations could probably be devised for all the autonomic phenomena; but one could not have much confidence in so elaborate a superstructure built on such slender foundation. It is safer to return to the general conclusion that the action of autonomic poisons *may* be chemical, and *may* be exerted on the more labile portion of the general molecule of the cells; there is really no evidence that even this is really the case.

c. The physical system of the cell as its receptive mechanism. The Nernst theory of stimulation, i.e., changes in the electrical potential of the cell, can be made the basis of interesting explanations for the receptive mechanism and its phenomena:

The double innervation with usually contrary response could be explained by the termination of the nerves at regions of different potential in the cell, which we shall call A and B. The contrasting regions could be nuclear and contra-nuclear; central and polar; membrane and sarcoplasm; exterior and interior—it is not necessary to settle this for the present. For the sake of illustration, we shall assume the nuclear and contranuclear regions, as these are undoubtedly at different potential. As the nerves develop in the embryo and grow out to make connections with the muscle or gland cells, they would naturally be directed to one region or the other of the cell, by this polar distribution of energy, or by other causes. It may be imagined that this direction would differ for the two systems of nerves, if these present different chemical or energy conditions—due perhaps to their different distance from their nutritive ganglion cell. The parasympathetic innervation could thus be attracted to region A and the sympathetic to region B— the nuclear and contranuclear region; the exterior and the interior of the cells etc. The relative importance of these two regions may be conceived to vary for different types of tissue, and with conditions, so that either the sympathetic or parasympathetic innervation may be dominant. It is also possible to imagine that in the cell of some regions conditions exist that reverse the directive forces, and thus account for reversed innervations; or that the directive force is so nearly neutral that both innervations connect more or less with one region, or with both regions.

The physical schema also offers a plausible picture of the direction of the response: Let us assume that approach to a certain electric equilibrium, say to the iso-electric point of the protoplasm, causes contraction of the cell, through surface tension, imbibition, osmosis or some such process; and that departure from this point causes relaxation. Increase of potential at region A would affect the electrical equilibrium of the cell in the opposite direction from increase of potential at region B of the same cell; and thus contraction or relaxation would ensue according to whether the nerve connected with region A or with region B. This would explain the usual reciprocal character of the two innervations.

It does not follow that stimulation of region A produces the same direction of response for all cells; it need not even be always uniform for any one cell. The response would be influenced by the electrical condition of the cell; just as the iso-electric point of a protein is either approached or receded by the addition of an acid, according as the original protein was alkaline or acid. This may be the key to the phenomena of inhibition by the dominant innervation; to paradoxic responses to nerve stimulation; to the effects of ion-ratios; to various colloid and anaphylactoid phenomena; and to the specificity of autonomic drugs.

These drugs may be conceived as acting chemically or physically on the cell in a large variety of ways; by producing precipitation or solution; changes of the ion-permeability of the cell membranes or of the protoplasm itself; changes in surface tension; changes in the size and surface charges of the colloids; changes in oxidation, reduction, cleavage or synthetic processes; direct chemical combination with the cell constituents, etc. Whatever the change, it must necessarily lead to a change of the distribution of potential within the system. The change produced by any particular drug must tend to react more upon one region than upon the other; and this results in more or less specificity. The high specificity of pilocarpin and epinephrin suggest that these act very predominantly at opposite regions, to produce the same changes of potential as nerve stimulation; but the phenomena of transposition suggest that they act somewhat throughout the cell, the dominant response depending on conditions, i.e., on "sensitization." Such conditions could be created by the simultaneous presence of both drugs; or by disturbing the energy distribution of the system by changing the ion-permeability of the cell wall by calcium or potassium, etc; or in anaphylactic sensitization perhaps by a change in the size of the colloid aggregates.

4. The Responsive Mechanism: The sharp separation of this from the receptive mechanism is of doubtful validity: the functions of excitability and contractility or secretion are so closely related that it is impossible to imagine a muscle that is excitable but does not contract; or one that contracts but it not excitable. The separation can only be made in a restricted sense; i.e., a muscle may be inexcitable for some particular kind of stimulation, but respond to others. In practice, the idea of receptive mechanism has been restricted to the transfer of nervous excitation and the excitability to specifically autonomic drugs; i.e., stimulations that may be conceived to act more especially on the contrasting A and B regions. Drugs like barium, caffein, the nitrites, the isoquinolin group, etc., seem to have less specific relation to these regions. They could be conceived as acting more generally throughout the cell; but it is again unprofitable to go far into details at present. It is difficult to imagine how they could leave the energy-distribution quite unchanged. Indirectly, at least, they must affect the irritability to all forms of stimuli.

The general physiological conditions of the cell must similarly influence its specific irritability, at least quantitatively. This is illustrated by the influence of tone and rhythmic activity on the response to autonomic poisons.

Space does not permit the further development of this subject, nor of many other incidental problems that are highly interesting, but which have less direct application to the basic phenomena that characterize the phenomena of autonomic drug response. It is a temptation to cite these at least in the bibliography; but even this would extend the review far beyond its allotted space. The following titles are therefore, confined to the material that is directly quoted in the review.

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THE LEUKOCYTES

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To Hewson we owe the discovery of the leukocytes, the early recognition of their importance in pathological processes we owe to Virchow, and to Ehrlich we are indebted for the development of a technique which made possible their differentiation. Ehrlich's classification of leukocytes, based on morphology and specific tinctorial reactions of protoplasmic granules, forms the base-line for almost a half-century of investigation of the varied hematological problems thus opened up. There has grown up in this period a literature of such tremendous volume that its review approaches the impossible and would in fact be undesirable, as, in the course of time, there has developed a certain stabilization of knowledge of the subject which renders many earlier discussions and opinions obsolete. My purpose therefore in this paper is rather to summarize or restate present-day opinions concerning leukocyte biology.

Any discussion of leukocytes must begin with a classification of the forms found in the circulating blood of the adult animal and the classification of Ehrlich is the one generally accepted, with, however, various reservations as to interpretation of forms and their relations. One must recognize three major groups of cells: the granular group of Ehrlich (the granulocytes), the lymphocytes, and the large mononuclear transitional group (the monocytes of Naegeli). The last two groups form the non-granular cells of the Ehrlich classification. That this grouping is valid and represents in all probability a fundamental variation in function of the different types of cells is clearly indicated by the fact that similar types of cells are found, not only throughout the vertebrate series of animals, but also quite generally throughout the invertebrates (Kollmann).

However, the claim of the Ehrlich school that the particular chemical reaction of the characteristic granules of the granular group of cells is necessarily a criterion of specific function has been challenged and possibly justly. Weidenreich in opposition to the Ehrlich view emphasizes the well-known fact that, while in man the granulation of the dominant polymorphonuclear cell has a neutrophilic reaction, in the rabbit the morphologically and functionally equivalent cell has an eosinophilic (pseudo-eosinophilic) reaction, in the guinea pig amphophilic, and in the rat and mouse granules are undemonstrable by use of the ordinary strains. It is further recognized that within a single species of animal the reaction of one type of granule may change in the course of its development. For example, in the guinea pig the granules in the developing eosinophilic myelocyte are basophilic in reaction (Downey). Even in the circulating blood in man, in a case of sharp eosinophilia (60 per cent of 13,000 leukocytes), I have noted basophilic granules among the eosinophilic and even cells in which granules of obvious eosinophilic size and character were entirely basophilic in reaction. Yet it must be admitted that the granules of the mature eosinophilic cells all show definitely an eosinophilic reaction. In spite of these variations in reaction, one must admit that in the circulating blood there are three distinct types of mature granular cell, the neutrophilic, eosinophilic and basophilic and that the distinct granules must in all probability be related in some way to the different functional activity of the cells. I am not willing to accept Weidenreich's conclusion that the neutrophilic is the only granule in the leukocytes of man which is of endogenous origin and the result of protoplasmic activity.

The application of the Romanowsky type of stain to the leukocytes early revealed an error in the Ehrlich designation of the lymphocyte and large mononuclear groups of cells as non-granular, hyalin types. The lymphocyte is shown to contain a few coarse,—at times rod-shaped—granules, with a metachromatic reaction, while the large mononuclear group shows a fine azurophile granulation. That these granules may be as important functionally as those of the so-called granular group of cells is not to be doubted.

This gives us then five distinctive types of granules in five separate and distinctive types of cells in the normal circulation of man; the polymorphonuclear cell with fine neutrophile granules; the polymorphonuclear (usually bilobed) cell with coarse eosinophile granules; the cell with irregular nucleus (often polymorphic, sometimes round) with coarse basophilic granules; the small lymphocyte with a few metachromatic granules; and the large mononuclear (or transitional of Ehrlich) with a fine azurophile granulation. There is no tendency in medical literature at the present time to regard these as other than independent forms,—end-cells—with no development from one type into another. The Ehrlich term "transitional" for the large mononuclear cell with indented or lobed nucleus, has long been recognized as a misnomer. The cell does not become a polymorphonuclear neutrophile, as was supposed. There has been a shifting of discussion from the question of possible relationship and transition between these mature cells to the question of relationship between their ancestor-forms, and, further, of the relationship of these stem cells to the ancestor of the red blood cell series and to endothelium. As it is universally agreed that the primitive parent of all blood cells is an undifferentiated embryonic mesenchyme cell, the moot point is the question as to how early or how late the totipotentiality (as far as the blood is concerned) of this cell is lost. Does differentiation take place early in embryonic life, or does there survive in lymphoid tissue (including the red marrow) until late in adult life or in fact throughout adult life, a cell that is still totipotential? This question is the Shibboleth which has divided the camp of the hematologists. One group, following the lead of Ehrlich, accepts the answer of an early embryonic differentiation of cells, and constitutes the so-called polyphyletic school. While this school recognizes the presence of immature, undifferentiated cells in the hematopoietic tissues of the adult, yet they would assign to such cells the power to develop along but a single line predestined from the embryonic period. These cells, then, morphologically indistinguishable from each other, are potentially wide apart. The monophyletic school, on the contrary, following the fundamental studies of Pappenheim, Maximow and others, recognizes in an indifferent free amoeboid mesenchyme cell of lymphocyte character, with basophilic granule-free protoplasm, the parent stem cell of all types of blood cells—a polyvalent cell, persisting in lymphoid tissues throughout life. The line of development of this cell depends upon the type of external stimulus and not upon inherent latent tendencies.

The embryology of a fixed tissue is difficult, but how much more difficult the determination of relationship in a group of cells which are not only capable of independent motion and early wander from their place of origin, but which also are soon caught in the whirl of a circulating medium. In addition to general recognition of origin from a mesenchyme cell, there seems to be agreement that in all animals the first blood formation takes place in the yolk sac. Beyond this point one is forced to conclude that there must be variation in different orders of vertebrates or that observation is of such difficulty that personal opinion weighs heavy in the conclusion, for even in the later embryological studies there is wide divergence of opinion.

Thus Stockard in a study of the living yolk sac of a Teleost, (Fundulus heteroclitus) finds that four distinct types of cells, endothelium, hematoblasts and two types of chromatophores, develop in the same environment from apparently similar wandering mesenchyme cells. His conclusion is that these cells must have been differentiated before they wandered from the point of their origin else the identical external conditions should have produced differentiation along but a single line. In further experiments, in which the heart's action and the development of a circulation was prevented by alcoholic intoxication, he finds that although vessels are formed, they contain no red blood cells and there is not the slightest evidence of red blood cell formation from endothelial cells. A further early differentiation or predestination he believes is indicated by the fact that the red blood cells arise in the posterior part of the embryo, while the leukocytes arise later and from the anterior region.

In birds, on the other hand, Sabin, working with living yolk-sac membranes, finds that there is first developed from the mesenchyme an angioblast, from which are derived not only endothelial cells but also red blood cells. The latter may also arise from more mature endothelial cells. The endothelial cells, further, proliferate, giving rise to large mononuclear cells of the blood and the clasmatocytes of the tissues. The forerunner of the granular leukocyte of the chick appears in tissues in close proximity to blood vessels and is at first not to be distinguished from a single angioblast. It is only surely distinguishable by its behavior after division. Two angioblasts cohere, leukoblasts separate. Sabin does not recognize a primitive common blood stem cell or "hematoblast" and apparently does not wish to identify her "angioblast" as such.

Danchakoff, however, studying embryonic chick material, concludes definitely that there does separate from the primitive mesenchyme a parent blood cell—the "hematoblast"—which develops into red blood cells if caught within a developing blood vessel, and into a leukocyte if it lies in a different environment, that of the extravascular tissues.

Thiel and Downey studying hematopoiesis in the spleen of the embryo pig, find that a "hematoblast" frees itself from the mesenchyme and develops into red blood cells chiefly in extravascular spaces. While few leukocytes are formed at any time in the spleen, they apparently arise from a similar or the same parent stem cell. Lymphocytes separate from mesenchyme cells directly without an intervening hematoblast stage.

It is difficult to reconcile such varied findings as these with the assumption of a similar type of development of blood cells in all types of vertebrates. One might perhaps better conclude it is another case of scales, feathers and hair. Gross variations in hematopoiesis and in mature cells are found in adult animal forms of the different groups. It is well known, for example, that in the adult bird, red-cell formation takes place intravascularly, while in the mammalian marrow it occurs definitely extravascularly. The loss of nucleus from the mammalian red corpuscle is another well-known deviation from the blood of lower vertebrate forms. The variation in type of the special granules of the polymorphonuclear leukocyte, seen among the members of the mammalian group, has already been referred to.

In spite of the lack of desired proof from embryological studies, there appears to have been a growing tendency to accept the unitarian or monophyletic doctrine for the origin of the blood cells, even in adult life. The polyphyletic doctrine, allowing in the adult only homoplastic origin of blood cells, like cell from like, seems too narrow to explain many pathological phenomena of hematopoiesis. It postulates too sharp a specificity of cells. The problem is practically the same as that with which one is confronted in the fixed connective tissue cells. One must apparently concede that the latter can not only lay down both white fibers and elastic fibers, but also, under certain environmental stimuli, can show a metaplasia and assume an osteoblastic function. The only difference between the assumptions for the two groups of cells is that in the fixed connective tissue cell the variation in function is manifested in a variety of extracellular products, while in the wandering blood cells it is indicated by an intracellular variation. It seems quite possible then that one may have to assume for the whole mesenchyme group of cells a greater plasticity or a more permanent possession of embryonic characters than is generally assumed or than is shown by epithelial structures. If the transformation of smooth muscle into the striated voluntary muscle type as claimed by Carey under certain experimental conditions is confirmed, we have further evidence of plasticity even in the more fixed tissues of the middle germ layer.

The cell for which this totipotentiality is claimed has received at the hands of hematologists a great variety of designations; the large lymphocyte, the indifferent lymphoid cell, the lymphoblast, the lymphogonien, the myeloblast, etc. It is a cell with large, pale-staining, vesicular nucleus and a scant rim of basophilic protoplasm without specific granulation—a young-looking undifferentiated cell. Such a cell is found constantly in the bone marrow and in lymphoid tissue. That a totipotential cell of this character is present, in small numbers at least, in the normal circulating blood appears to be the simplest explanation of such phenomena as the occurrence of red marrow in metaplastic cancellous bone in the aorta, as described by the author, and the experimental production of red marrow in association with metaplastic bone in the necrotic kidney of the rabbit as described by Maximow. Yet such a circulating cell has not been positively identified unless one assumes with Maximow that any of the smaller and larger lymphocytes may grow into this indifferent lymphoid cell and that this latter may then show metaplastic development. I have been inclined to regard the smallest lymphocytes as end cells and to assign embryonic characters to the large mononuclear cell with round nucleus and very basophilic protoplasm.

In certain pathological blood pictures and, in particular, in the acute leukemias of marrow origin, the circulation is fairly flooded with primitive cells. One has in the acute myelogenous (myeloblastic) leukemia an unlimited proliferation of this primitive cell without differentiation. Accordingly, there is lacking in the circulation every element that might be derived from it. Red blood cells show a constantly diminishing number (an aplastic anemia); granular leukocytes and blood platelets are lacking. Only myeloblasts and lymphocytes represent the leukocytic content. In the chronic splenomyelogenous (myelocytic) leukemia one has an equally unlimited proliferation but with partial differentiation, and the blood stream is flooded with myelocytes, nucleated red blood cells, platelets (small and large) and even with the megalakaryocytes. By contrast again, in pernicious anemia differentiation is toward the hemoglobin-containing cells, and marrow leukocytes and platelets show diminishing numbers as the disease progresses. I am aware that the "crowding out" theory has been advanced to explain the phenomena just cited, but it seems less satisfactory. The monophyletic theory seems eminently satisfactory even though it offers some difficulties in addition to the lack of absolute proof. Danchakoff objects that indifferent cells in marrow tissue should be subject to the same stimuli and thus differentiation should proceed in the same direction in all. I doubt the validity of the premise upon which the objection rests. Evidence tends much more toward the view that physical and chemical conditions are not uniform throughout even small masses of tissue and probably not throughout even the colloid mass which constitutes the body of a single cell. Varying distances from the source vary the intensity of normal and toxic stimuli, oxygen tension and the like.

The assumption then of the totipotential cell simplifies the consideration of hematological problems if one can avoid the complicated schemes of development and relationship propounded by certain advocates of the monophyletic theory, notably Pappenheim. From this indifferent cell there is differentiated, as I have suggested elsewhere, a sufficient amount of varied marrow and lymphoid tissue in the body to maintain a fairly constant circulating number of red and white cells. Production keeps pace with destruction, as is shown by the constant ratio manifested in the circulating blood. While there is a rather wide variation in the normal number of leukocytes and the percentage of different forms as seen in the differential count in different people, for an individual in health the number and the differential picture are fairly constant. One may still say the average normal total leukocyte count is 7500 per cu. mm. (7300, Miller's figure for 280 cases). However, the author was surprised, in making differential counts on supposedly normal students at Wisconsin University, to find the proportion of the various forms of cells quite different from that given by Ehrlich. The 70 to 72 per cent of neutrophile leukocytes of the Ehrlich data was strikingly above the average given in the series of the author's counts (54.6 per cent) in which a Romanowsky type stain was used instead of the Ehrlich triple stain. Miller's repetition of the work on a much larger scale gives an intermediate figure of 62 per cent, in counts with Wilson's stain. I am inclined to accept Miller's figure not only in view of his larger material, but also as a result of my long-continued study of the changes produced in the leukocytic formula by low grade chronic non-disabling infections, particularly of the upper respiratory tract which are particularly prevalent in the locality in which my counts were made. Latent "childhood" tuberculous infection is quite apparently the modifying agent in some of the counts given.

The occurrence of a normal individual leukocyte formula raises immediately the question of its importance. Has it physiological significance and if so what significance? In answer to the question, it would seem that at the present time we have no evidence of what might be called a purely physiological function for the leukocytes. As far as it is known, their function may be said to be pathological. At least it is protective, and less physiologically protective than that of skin. It is rather difficult to accept this conclusion when one considers that the leukocyte is a much more primitive cell than the physiological red cell, and appears much lower in the animal scale than the latter. Yet one must recall that protection of the body from attack and invasion by alien and enemy forms is needed by the invertebrate as well as by the vertebrate animal. In man and the higher animals all the exposed surfaces, inner as well as outer, are crowded with organisms, many of which are pathogenic and capable of invading the body or harming it by toxin production. Finding evidence in certain acute infectious diseases (measles, influenza) that pyogenic complications occur commonly when the total number of polymorphonuclear neutrophile leukocytes falls below a certain level per unit volume of blood, it has seemed justifiable to conclude that their number per unit volume is normally maintained at a protective level against these same pyogenic organisms present on mucous membranes. Bunting and Huston have described in normal animals the daily emigration of billions of lymphocytes upon the mucous membranes and in particular upon that of the intestine. It would seem that they fulfil their functions there normally. As the lymphocytes are not phagocytic it is suggested they act as affixers of toxins, and thus contribute to the immunity which the intestine has to the contained organisms. Limiting the function of these leukocytes to that of protection may be simply the expression of ignorance, yet it would appear to measure up to the present knowledge we have concerning them.

For further consideration of the leukocytes it would appear best to take up each type of cell separately.

The neutrophile leukocyte. Neutrophile leukocytes are found in the marrow of the human fetus between the fourth and sixth month. They are also found in connective tissue elsewhere, especially about the thymus and in the liver (Browning). In the sheep, Goodall finds an occasional leukocyte in the liver in the 2 to 3 cm. embryo; in the 4 to 5 cm. embryo leukocytes are fairly numerous about the thymus; above 5 cm. there is beginning hematopoiesis in the marrow which becomes active at 10 cm. After birth, leukocyte formation in the higher animals occurs normally only in the marrow. The mature neutrophile, with its characteristic nucleus, is a development through well-recognized changes from the myelocyte with round vesicular nucleus and a neutrophilic granulation. The myelocytes arise both homoplastically by mitosis in similar granular cells, and heteroplastically by the development of granules in a primitive cell with hyalin protoplasm. I have emphasized the fact that the marrow cells are grouped in extravascular centers, in which the cells develop from the center toward the periphery, so that the mature cell comes to lie on the outside, adjacent to venous sinuses. The mature neutrophile, which is the most actively amoeboid of the circulating cells, apparently enters the blood stream through its active efforts and under some unknown chemiotactic influence. Equally unknown is the regulatory mechanism of the supply to the circulation. A normal marrow apparently contains a considerable supply of mature leukocytes. This is seen in sections of red marrow. It is also indicated by unpublished experiments in this laboratory which show that in the rabbit so slight a stimulus as the injection of 1 cc. of normal salt solution in an ear vein will call out a total of 300,000,000 neutrophiles (pseudo-eosinophiles) in one hour. This time interval is too short for the increase in circulating cells to represent other than an increased out-pouring. It is not apparent why but a certain proportion of these cells enters the blood stream normally and the rest remain in the marrow as a reserve.

As the neutrophile enters the blood stream its nucleus usually shows three lobes with connecting chromatin bands. Apparently as a result of ageing, the lobes increase in number to 4, then to 5 and even to 6, as emphasized by Arneth, who has constructed definite formulae, indicating the degree of marrow activity. The length of life of the neutrophile within the circulation and its ultimate fate have not been absolutely determined; Weiskotten and Steensland, by argument not entirely free from criticism, have concluded from leukocyte curves obtained in benzol poisoning in the rabbit, that the neutrophile lives 3 days in the circulation.

The granules in the protoplasm of the neutrophile are held to be endogenous and the result of protoplasmic activity, even by Weidenreich, and they are the only leukocytic granule so accepted by him. That any feature of its functions is displayed while in the normal circulation we have, as stated earlier, no evidence. The part played by the cell in pathological processes, in the coagulation of blood, in the digestion of proteid by its proteolytic enzyme, active in an alkaline medium (Opie), and in the ingestion and destruction of bacteria, in particular of the so-called pyogenic group, is too well known to need more than mention. It may be said however that it is becoming more evident that the neutrophile is not an active defensive agent against all infecting organisms, but that in several acute infections, notably typhoid fever, measles and influenza, and in at least one chronic infection, tuberculosis, there is a marrow inhibition, with a diminished number of neutrophiles in the circulation. Defense seems to lie with the lymphoid and mononuclear groups of cells. Even in pyogenic infections, the endothelial phagocyte is found to play a part almost if not quite as important as that of the neutrophile.

The eosinophile leukocyte. Eosinophile leukocytes are found in the fetal tissues as early and possibly earlier than the neutrophile. Browning found them in the walls of the hepatic and umbilical veins in a fetus of 10 weeks and in the marrow at 3½ months. The eosinophile of the adult circulation is a cell of marrow parentage, and like the neutrophile is descended through a series of myelocytes which may have had either a homoplastic or a heteroplastic origin. The theory championed by Weidenreich among many others that the eosinophile granule represents not an endogenous formation but exogenous material related to hemoglobin or its dissociation products, seems so far removed, as even a plausible deduction from the easiest observations and experiments, as to be an absurdity. The whole question of the eosinophile has been recently reviewed by Downey and by Ringoen, who give abundant proof of the opposing theory, if it were needed. With the early disposal of the theory that the eosinophile granule was but a later stage of the neutrophile, and with the demonstration of the endogenous nature of the granule one may recognize in the eosinophile a cell of specific character. Whether or not all eosinophiles are of marrow origin must apparently remain a question still to be discussed and investigated. Ringoen, apparently, is convinced that eosinophiles are formed in the hemo-lymph nodes of normal sheep, as well as in the marrow. In a study of hundreds of human lymph nodes I have never seen evidence of eosinophile production in them. Emigration of eosinophiles from the blood stream into lymph nodes is of extremely common occurrence, especially in pathological conditions, in which there is a destruction of lymphocytes within a node.

The function of the eosinophile cell is problematical. It is ordinarily not phagocytic. While its number in the normal circulation is small, under stimulation of the marrow and reaction of the latter the number in the blood may be augmented more than a hundred fold, with every evidence of emigration of vast numbers from the circulation. The cell appears to share in the defense against various animal parasites. In addition it is found in the tissues, in inflammations of the skin and mucous membranes of varied etiology and in the neighborhood of tumors of great variety. Eosinophilia is one of the phenomena of anaphylactic shock. Ringoen sees a possible chemiotactic influence in the products of autolysis of cells as suggested by Fessinger, as an explanation for the appearance of the cell under this great variety of circumstances.

The basophile. The basophilic cell of the blood was at first designated a "mast cell" by Ehrlich on the supposition that it was identical with the "mast cell" of the tissues. The work of Maximow, Weidenreich, Pappenheim and others has shown such marked differences between the two cells that they must be recognized as of two distinct orders with a basophilic (metachromatic) reaction to granules in their protoplasm as the only common feature. According to Maximow there is to some extent a ratio between the number of basophiles in the blood and that of the tissue mast cells. Animals with many circulating basophiles have few in the tissues and the contrary is also true. The status of the circulating basophile has apparently not been definitely agreed upon. Pappenheim, Weidenreich and others consider it not a true granulocyte but a degenerating cell, the granules representing products of degeneration. Maximow and Downey, among others, are in accord with the original Ehrlich conception that it represents a specific granular cell of bone marrow origin. They describe homoplastic and heteroplastic development of basophilic myelocytes from which the circulating cell is derived. Physiological observations seem to confirm the latter point of view which was arrived at by the authors mentioned from a study of tissue sections. Circulating basophile leukocytes are increased relatively and absolutely in at least two conditions in which one has evidence of increased marrow activity, chronic myelocytic leukemia, and polycythemia vera. They are also increased in chronic inflammations of the accessory nasal sinuses. It would seem that they must be regarded as specific cells; but we are totally in the dark as to their function.

The large mononuclear and transitional of Ehrlich. These largest cells of the circulation, the so-called large mononuclear leukocytes or monocytes have been the subjects of much discussion relating to their origin, to their relation to other cells, and to their function. In the circulating blood we find, normally, large cells with two types of nuclei. The one has a large round or oval nucleus and a very basophilic protoplasm and forms in the author's large series of differential counts upon human blood, but 0.2 per cent of the circulating leukocytes, upon the average. The other has a nucleus of varied shape, at times merely indented, more commonly trilobed, at times S-shaped, and there is an abundant protoplasm somewhat basophilic and containing fine azurophile granules with Romanowsky-type stains. This cell is more numerous than the former and constitutes approximately 6 per cent of the circulating leukocytes. It is this cell to which Ehrlich gave the name "transitional" on the mistaken theory that it represented a stage in the development of the neutrophile leukocyte. Ehrlich was uncertain whether its origin was from the spleen or bone-marrow but considered the latter most probable. For many years this cell was considered to be the makrophage of Metchnikoff, and thus concerned in phagocytosis. Mallory and his students have spoken of it as the "endothelial leukocyte" on the supposition that it is identical with the phagocytic endothelial cells freed from the lymph sinuses and hem-vascular walls. In her recent article in these REVIEWS Sabin practically accepts this view as a result of her investigation of the growing vessels in the yolk-sac of the chick. She finds multiplication of endothelial cells which wander both into the lumen of the vessel (monocytes) and into the tissues (clasmatocytes). In adult animals there is also shown to be multiplication of endothelial cells and their separation from the vessel wall and entrance into the circulation (Evans, McJunkin, Simpson et al). There is no doubt that under proper stimulation experimentally or under certain pathological conditions endothelial cells may be found in the circulating blood. Simpson in Evans' laboratory has shown that with the repeated injection of colloidal dyes into animals these cells occur in showers and may constitute 90 per cent of the leukocytes in the right heart, yet at the same time constitute but 0.1 per cent on the left side. Yet this is not proof, as some authors seem to contend, that endothelial makrophages and large mononuclears are identical.

The application of vital staining to the leukocytes, both intravitam and on the slide (supravital method), it has been hoped would settle this question. It is perhaps unfair to depend entirely upon a so-called biological method to determine cell-relationship. Because two cells ingest or absorb the same type of dye-stuff would not seem an absolute proof of identity. It can be shown experimentally that both endothelial cells and polymorphonuclear neutrophiles will engulf staphylococci. They are thus far biologically related. Likewise large mononuclears and endothelial cells will behave alike toward certain dyestuffs, differently toward others. The results and interpretation of results vary in different hands. Without pursuing the controversy at length, I think the fairest statement of what the present day point of view must be, is the conclusion of Miriam Simpson, that while her extensive work with vital stains "points strongly to the biological affinity of these two interesting cell types, it cannot be said to show an identity or that one may be transformed into the other in the free or circulating blood stream. This evidence is in consonance with what we know about a similar lack of transformation of one type of leukocyte to another in the blood current, our information tending to bring the conviction that a cell once shed into the stream henceforth usually undergoes no significant transformation."

My own study of the leukocytes of the circulation in normal and pathological states has led to the conclusion that the large mononuclear with the round nucleus is an immature cell and is found in the normal circulation largely as an accident. In its mature form it is the transitional cell of Ehrlich. The cell of this type, found normally, is a marrow cell and is increased in marrow reactions of a proliferative character. Yet in certain pathological conditions with unusual stimulation of the lymphoid tissue all the large mononuclears found in the blood are not of this origin. The large mononuclear group becomes heterogeneous and includes many cells of lymph gland origin. This is true not only of the acute lymphatic leukemia picture, but also of the blood in such infections as smallpox and measles. Endothelial cells may also be found in the blood in these diseases.

The lymphocyte. It is quite generally agreed that most, if not all the lymphocytes of the circulating blood are derived from the lymphoid tissue in the body and reach the circulation through the large lymph channels. This would seem abundantly proved by the work of Rous, Carlson and Davis, and Bunting and Huston. I find, however, as late as 1908 Gulland and Goodale expressing the opinion that most if not all of these cells of the circulation have their origin in the bone marrow. Although extramedullary lymphoid tissues furnish the main source of lymphocytes, it is generally accepted that typical areas of lymphoid tissue are found in the marrow, and that this tissue may furnish some cells to the circulation.

In the normal circulating blood one finds smaller and larger lymphocytes. The larger have the same type of nucleus, but this is surrounded by more protoplasm of a less basophilic reaction, containing usually more coarse granules. It seems safe to assume that this represents but an older stage of the smaller cell. It is not to be confused with a larger type of lymphocyte with very basophilic protoplasm which appears in the circulation under intense stimulation of the lymph glands in certain infectious diseases, notably measles and smallpox, and also in the acute type of lymphogenous leukemia. This last cell is present in normal lymphoid tissue but does not normally appear in the circulation. Certain unpublished observations of the author and Mr. Huston show that if one makes impression preparations from lymph glands of the rabbit and measures the cells, while all gradations are found, the greatest number of the cells group themselves into three major groups; the largest cells, corresponding to the lymphoblast, have a diameter of from 18 to 20 μ; an intermediate group which varies between 10 and 12 μ; and the groups of smallest cells between 5 and 7 μ. Mitotic figures were found in cells of the first two groups, but were not seen in the smallest cells. This suggests a development similar to that of the red cell in which the intermediate red cell of Howell stands between the megaloblast and the normoblast.

It is generally accepted that it is by mitosis in the lymphoblasts of the germinal centers of the lymph glands that the development of the lymphocyte is inaugurated. This intermediate sized cell with its capability of mitotic division and thus of a geometric progression in the multiplication of cells, has not been generally recognized. It may, however, account for the undue number of small lymphocytes apparently produced by mitosis in a few lymphoblasts.

There is still uncertainty as to the embryology of the lymphocyte and even of the post-natal origin of the cell. Some, as Danchakoff, maintain the development of the lymphocyte from a hemocytoblast, the primitive blood cell cut off from the mesenchyme. Others, as Thiel and Downey, maintain that small lymphocytes are cut off directly from the mesenchyme cells without the intervening hemocytoblast stage. Sabin's study of the lymph nodes indicates the possibility of this latter mode of development without, in her opinion, proving it. Weidenreich and Downey hold it definitely proved that in post-natal life, the reticulum of the lymph glands, the fibroblastic tissue of the omentum, and possibly also that of the loose connective tissue retain the capactiy of the embryonic mesenchyme to liberate free lymphoid cells. The orthodox view holds the lymphoblast of the germinal center as the parent of the lymphocyte in the adult animal. The necessity of postulating a tissue lymphocyte has largely departed with the general recognition of amoeboid motion in the lymphoid cell, yet complete harmony of views on the origin and relation of the cell cannot be expected at the present state of our knowledge.

The work of Rous, Carlson and Davis, and others has shown that more lymphocytes enter the blood stream during 24 hours than are present in the circulation at any one time. Bunting and Huston, in searching for a solution as to what becomes of this large number of cells in normal animals, have found that they not only leave the blood stream but also the body finding their way out onto the mucous membranes and in particular onto the intestinal. Their normal function is apparently fulfilled there. This function is unknown. If it be protective against the bacterial content of the intestine, it cannot be of a phagocytic nature, as the lymphocyte is not a phagocyte. It is suggested that it may be antitoxic or, in other words, that the lymphocyte may contribute to the immunity of the intestine to its contained bacteria by affixing toxins. During the recent years the lymphocyte has gained increasing consideration as a defense cell in pathological processes. The work of Murphy and his associates has demonstrated that it is an important cell in the defense of the body in such infections as tuberculosis and against cancer. The nature of this defense, again, is unknown. Owing to its lack of phagocytic power one seems left with but the possibility that the lymphocyte acts by affixing toxins and if not destroyed by that toxin, by the production of antibodies. The normal distribution of lymphoid tissue in the body, the position of lymphoid cells in pathological tissues all speak for such a theory.

It seems unnecessary to further condense this inadequate summary of hematological problems into a formal set of conclusions. In the present state of our knowledge, judgment passed on many of these problems would be premature and would have only the weight of opinion. It must be left to further investigations to determine in full the life history and the functions of the different leukocyte forms.

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THE PRESENT STATUS OF THE FUNCTIONS OF THE THYROID GLAND

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We probably possess a more detailed knowledge of the embryology, anatomy, physiology, chemistry and pathology of the thyroid than of any other gland. But as regards its interrelations with other tissues,—a vastly greater and more important field,—it can truthfully be stated we are only at the beginning of any definite knowledge.

No attempt will be made to refer to or review all the literature relative to thyroid physiology that has appeared during the last decade. Excellent reviews of this type are available in an article by Vincent (1) in 1911 and in certain textbooks, notably Biedl's (2). Rather a critical summary of the present status of certain phases of our knowledge of this gland and some of the important steps involved in acquiring it as they appear to the author will be attempted. Owing to the recent great popular interest in endocrinology (one is tempted to say endocriminology) the thyroid gland has suffered with the rest, though perhaps less than its sister glands, from loose speculation. This, however, is only because we possess a greater number of unchallengeable facts relative to the thyroid.

ANATOMY. *a. Ancestry and embryology.* Owing to the survival of one class of vertebrates—the Cyclostomes—it has been possible to establish the ancestry of the thyroid of all higher vertebrates as a direct metamorphosis of the endostyle organ. The endostyle organ is an elaborate ventral mid-line pharyngeal gland in Tunicates, Amphioxus and Ammocoetes (3). In Tunicates and Amphioxus it opens into the pharynx through a groove extending the full length of the organ (5). In Ammocoetes the opening into the pharynx is reduced to a large duct (4). During the metamorphosis of Ammocoetes the endostyle organ undergoes atrophy with complete loss of three of its specialized types of epithelium including the duct, and the ductless thyroid follicles of the adult are formed from one persisting type of endostyle epithelium (6). These cells sometimes retain their cilia after metamorphosis. The ductless thryoid follicles in Cyclostomes arise solely from the endostyle organ. Studies in the embryology of the thyroid of fishes, amphibians, reptiles and birds have shown that it also arises solely from a median single ventral tubular downgrowth of the pharyngeal endoderm in or slightly anterior to the first aortic arch (8), (9). This symmetry and uniformity of development was believed to be departed from in mammals owing to the discovery by Stieda (10) of the so-called lateral thyroid anlagen. These lateral thyroid anlagen were believed to give rise to the lateral thyroid lobes and to be developed from the fourth gill pouches, or more accurately in man from the rudimentary fifth. The view that the thyroid in mammals arises from three separate anlagen persists and still appears in many textbooks. Recent studies of the fate of the so-called lateral thyroid anlagen, post-branchial bodies, or better, ultimo branchial bodies, show that they are only atrophic remnants from the fifth pair of gill pouches which, during development, may become attached to or even embedded in the lateral thyroid lobes, but take no part in the formation of thyroid tissue (11), (12). This solution of the origin of the mammalian thyroid makes it possible to interpret many of the pathological changes and developmental defects of the thyroid, and makes the origin and development of this gland uniform throughout all vertebrates (7), (13).

b. Gross and microscopic. Morphologically the thyroid is one of the simplest of body tissues and resembles the lung more closely than any other tissue. Indeed, there are many embryological, anatomical physiological and pathological relations between the thyroid and the lungs the study of which has added much to our present-day conception of interrelationships. The thyroid tissue is one of the most labile tissues in the body—capable of rapid overgrowth and of equally rapid involution. Its wide range or cycle of morphological changes makes it possible to detect easily morphological changes which if interpreted in terms of function are but little removed from the normal, but if compared with similar types of morphological changes in other tissues less well endowed with the capacity for growth have frequently resulted in drawing wholly unjustified conclusions regarding the alteration of function.

The thyroid has only one known cycle of cell changes and it tends to repeat this cycle in response to all stimuli involving increased functional activity.

The normal human thyroid weighs between 20 and 25 grams and does not exceed 0.35 gram per kilo of body weight (36), (14). Statistical data indicate that the thyroid is slightly larger in females per unit of body weight. The gross outline of the thyroid is quite variable, the greatest variation occurring in the isthmus or pyramidal process (16). In the strictly normal human thyroid the isthmus is a band of tissue from 1 to 2 cm. in width and from ½ to 1 cm. in thickness connecting the two lateral lobes across the trachea anteriorly just below the level of the cricoid cartilage. The presence of a pyramidal process and thyroglossal stalk must be considered as due to incomplete absorption of this portion of the thyroid tract which normally takes place between the fifth and eighth week of fetal life. In severe endemic goiter districts approximately 95 per cent of human thyroids have well-formed pyramidal processes and thyroglossal stalks frequently continuous with the foramen cecum (15). Similar variations are seen in animals; thus, in congenital goiter of dogs and sheep the thyroid lobes are usually joined by an isthmus while normally in these animals the isthmus undergoes absorption before birth.

The gland is invested with an outer fascia which strips readily and exposes a slightly lobulated smooth surface formed by the inner or true capsule. This is thin and translucent. Thickened portions of this capsule (trabeculae) extend into the gland, support the blood and lymph vessels and give it a slightly lobulated appearance (34), (35). The color of the normal thyroid varies from a pale translucent amber red to a bright translucent amber red. The normal gland is of firm consistency and made up of closely packed circular or oval closed alveoli filled with a glairy honey-colored viscid globulin—the so-called colloid, which gives to the thyroid its specific characteristic, chemical as well as physical. The thyroid unit or alveolus is similar in all vertebrates (30), (31), (32). In man these alveoli vary from 0.1 to 0.5 mm. in diameter and are lined with a single layer of low cuboidal epithelium (columnar epithelium always indicates hypertrophy) (17). The older observers (Langendorf (18), Biondi (19), Hürthle (20) and others) recognized two types of gland cell, the so-called chief and colloid cells. The former are more granular while the latter contain in addition vacuole-like globules filled with a thin fluid which some have considered as a thyroid colloid, similar in many respects to that contained in the alveolar spaces (33).

In recent years a great deal of attention has been paid to the finer specialized cytoplastic structures especially the mitochondria and the reticular material or Golgi apparatus (27), (28), (29). Cowdry (21), (22), (24) has reviewed the literature of both these subjects. Mitochondria (Altmann's granules) are present in all thyroids. They are very rare in the fetal thyroid and increase with age (23). Their number also varies with the size of the cell. Their lipoidal nature distinguishes them from secretion granules with which they were long confused. They can be correlated with functional activity as well as with cell growth. Bensley noted that the more active the cell the more numerous were its granules. Some authors, notably Goetsch (25), have in addition attempted to establish a relation between the pharmacological activity of the cell's secretion and mitochondria, particularly in exophthalmic goiter. This view has not received much support for the reason that the very actively hyperplastic columnar cells seen in the thyroids of myxedema are equally rich in mitochondria. They seem closely related in the functional activity of the cell but unrelated to the pharmacological value of the cell's secretion. In the thyroid cell, mitochondria are most numerous in the region between the lumen and the nucleus while in ordinary gland cells like the salivary or pancreas these granules are most numerous in the region between the nucleus and the basement membrane. Bensley pointed out that this was probably due to reversed polarity. Recently Cowdry has studied the thyroid cells of the guinea pig, using the reticular material or Golgi apparatus as an indicator of polarity. He found that the reticular material of the guinea pig thyroid cell was located in the region between the nucleus and the base of the cell in about one cell out of every five hundred, i.e., reversed, and accepts Bensley's (26) explanation that it indicates the ability of the thyroid to secrete either toward the bloodstream or toward the alveolar lumen, and this reversed secretion (toward the lumen) comes into play when the thyroid secretion is being produced in excess of the body needs. Reversed polarity has been observed only in the thyroid gland and as the ancestral thyroid (endostyle organ) was an external secreting organ it suggests that the reversed polarity is a relatively recently acquired characteristic to meet a change in function.

Lymphoid tissue, represented by scattered small foci in the stroma, is normally present in the thyroid (37). Under certain conditions associated with general overgrowth or persistence of lympoid tissue, as in status lymphaticus, Addison's disease and exophthalmic goiter, this lymphoid tissue may undergo an extraordinary hyperplasia with the development of well-formed germinal centers.

Accessory thyroid tissue other than that found along the thyroglossal tract occurs with great frequency. In dogs and cats accessory masses may be demonstrated in upwards of 90 per cent. The most frequent sites are in the thymus gland and in the region of the arch of the aorta.

In man there are normally small groups of undeveloped thyroid cells lying in the stroma between the alveoli. These have been spoken of as cell rests and their relation to the development of thyroid adenomata was first pointed out by Wölffler (43). It is believed that the thyroid anlage is capable of and actually produces during embryonic life many more cells than are usually required for functional needs. Under ordinary conditions, as in the case of striped muscle fibers during fetal life these thyroid cell rests undergo gradual absorption beginning in intrauterine life and continuing after birth. On the other hand, in the presence of a stimulus for increased thyroid activity, these undeveloped rests respond with growth and become the adenomata which are almost universally present in and an integral part of endemic human goiter. Thyroid adenomata are exceedingly rare in the lower animals.

These adenomata are highly variable in size, number and structure. One recognizes types of adenoma composed of closely packed small undistended alveoli—so-called fetal adenoma, and also types with well differentiated large colloid-containing alveoli and finally all gradations between these. Morphologically different types may be present in the same gland. It is believed these variations in morphology depend upon *a*, the stage of differentiation of the cell rest from which the adenoma arose; and *b*, the degree of differentiation occurring during its growth and involution. Adenomata are capable of taking up iodin from the circulation and of elaborating the iodin-containing hormone, thus differing sharply from thyroid carcinoma. The more differentiated types as regards evidence of functional activity approach that of normal thyroid while the fetal types may or may not be able to take up iodin even after the prolonged administration of iodides (44), (45).

c. Circulation. The thyroid has a very large blood supply variously estimated from 3.5 to 5.9 cc. per gram per minute (66), (67). Obviously in a tissue with such wide variations in functional activity correspondingly wide variations in the blood supply must be present. Control studies should be made with standardized thyroid and the thyroid happens to be the only tissue which at present can be standardized. This is accomplished by iodin administration. The thyroid arteries form rich anastomoses on the surface but none in the depths of the gland (40). Schmidt (38) and Horn (39) have described endothelial buds or "knospen" in the arterioles which may act to reduce the speed of the blood flow by a baffle-board effect. This also reduces the pulse-wave effects which in a gland with such a short and wide capillary path would otherwise easily pass through to the veins, especially in the marked hyperplasias. The thyroid gland can be perfused readily at a pressure of 15 to 20 mm. Hg. while the kidney requires from 70 to 80 mm. Hg. pressure. The capillary network surrounding each alveolus is comparable to the capillary network surrounding the lung alveolus. The thyroid is also richly supplied with lymphatic vessels which are collected for the most part into two major channels which leave the gland with the large veins (41). The thyroid hormone can pass directly into the blood stream, as has been shown by Rogoff and Goldblatt (42). There is no evidence except the older morphological evidence that it may also be discharged into the lymphatics.

d. Innervation. The anatomical studies of Berkeley (46), Anderson (47), Rhinehart (48) and others have shown that the thyroid is richly supplied with nerves, all of which are believed to belong to the sympathetic system. These nerves leave the spinal cord between the second and seventh thoracic segments and pass upward to the middle and superior cervical ganglia from whence they are relayed to the thyroid *a*, directly along the blood vessels, or *b*, indirectly through the superior laryngeal and possibly the recurrent laryngeal nerve. These nerves are distributed both to the perivascular tissues and to the bases of the gland cells—the latter have been recorded by anatomists as possible secretory nerves (56), (56a). The gland is richly supplied with vasomotor nerves both constrictor and dilator (54), (55). The question of secretory nerves has attracted much attention during the last decade and still remains undecided. All the evidence is indirect. Asher and Flack (49), (50) first showed that in rabbits the blood pressure response to a given dose of adrenalin was greater after electrical stimulation of the superior laryngeal nerve with intact thyroid than before such stimulation. They also showed that after thyroid removal, stimulation of the superior laryngeal nerve did not modify the blood pressure response to a similar dose of adrenalin. Oswald (51) obtained similar results by injecting iodothyreoglobulin instead of stimulating the thyroid nerves. Levy (52) has confirmed Asher and Flack's work on cats. Epinephrin produces all these effects and also markedly raises the heat production in the absence of the thyroid gland. The difference is only one of degree. Von Cyon (53) has criticized Asher and Flack's work by pointing out that where blood pressure is used as an indicator, many factors particularly the degree of anesthesia must be carefully controlled, and also one must distinguish between goiterous and non-goiterous rabbits for this kind of work. Rahe, Rogers, Fawcett, Beebe and others

(58), (59) have shown that the iodin content of the thyroid may be reduced by prolonged faradic stimulation of the cervical sympathetic. Cannon and Cattell (57) showed that there was an alteration in the electrical potential of the gland on stimulating the cervical sympathetic. No such effect was observed when the vagus or sciatic nerves were stimulated or when the systemic blood pressure was changed. They interpreted this as evidence of secretory innervation.

All these methods are highly indirect and complicated and the results are not susceptible of complete analysis at present. Other more direct proof must be offered before one can accept the doctrine of secretory nerve control of the thyroid secretion. That some sort of nervous control whether secretory or regulatory exists is probable on the grounds of analogy. Whether this regulation is dependent on specific nerves or on specific chemical changes acting through a more general regulatory nervous mechanism is still to be demonstrated. On the other hand, there is very direct evidence that specific nerve endings are not necessary for thyroid tissues to show many evidences of variation in secretory activity. Thus, by autografting the thyroid in widely separated parts of the body it has been shown that such transplanted thyroid wherever located shows chemical and morphological changes paralleling those of the non-transplanted tissue (60). If the transplant is undergoing hyperplasia, the non-transplanted portion is also undergoing changes similar in nature and degree. The administration of iodin inhibits hypertrophy alike in the transplanted and non-transplanted thyroid. In 1914 Cannon (61), utilizing Langley and Anderson's (62) method of anastomosing motor and sympathetic nerves, united the anterior root of the phrenic with the peripheral end of the cervical sympathetic in the cat and reported the occurrence of symptoms closely resembling exophthalmic goiter—tachycardia, emaciation, increase in metabolism to plus 100 per cent. He ascribed these phenomena to the bombardment of the thyroid with impulses discharged through the phrenic stump. These results have neither been confirmed by others (63), (64), (65) nor repeated by Cannon.

PHYSIOLOGY. *a. Effects of thyroid removal.* Our knowledge of the physiology of the thyroid may be said to have begun with Gull's report in 1874, *On a Cretinoid State Supervening in Adult Life in Women* (74). Prior to its publication, the function of the thyroid was either speculative—that it aided in the formation of erythrocytes, that it acted as the vascular shunt for the cerebral circulation, that it neutralized toxins, that it served only to give form to the neck, etc.,—or was hopelessly confused with the function of the parathyroids. It was not until Gley in 1891 (75) rediscovered the parathyroids that any real differentiation of the functions of the thyroid and parathyroids was possible, and it was some twenty years after this that their functions were finally separated to the satisfaction of all workers.

Independent observations reported by the Brothers Reverdin in 1882 (76), and more clearly by T. Kocher in 1883 (77), on the effects of total thyroidectomy in man for goiter established the first experimental confirmation of Gull's clinical-pathological observations. Some of these operated human cases developed parathyroid tetany and died, while others developed during the next thirty to sixty days a cachexia which these surgeons recognized as similar to that described by Gull. The Reverdins designated the symptoms complex as operative myxedema (Ord (78) in 1878 having given the name myxedema to the condition described by Gull because his chemical examinations indicated there was an increased mucin formation in the thickened subcutaneous tissue) while Kocher called it *cachexia strumapriva.* Horsley (79), (80) in 1884, working with monkeys, observed a few instances of cachexia strumapriva; most of the monkeys developed tetany. Other observers carried out similar experiments on rabbits, sheep, goats, dogs, cats and guinea pigs. Those working with rabbits claimed that thyroidectomy was usually without noteworthy effect, while those working with carnivora usually obtained parathyroid tetany. The experimental thyroidectomies before 1891 in general added confusion rather than facts to the function of the thyroid. Since 1891 many species of mammals have been subjected to thyroidectomy using standard surgical technique and excluding the parathyroid factor. The most striking effect of thyroidectomy is a reduction in the total metabolism. In cats and rabbits this decrease begins usually in from six to eight days after thyroidectomy and reaches its lowest level between the twentieth and thirtieth day (81), (82). This low level of metabolism may be maintained for years (rabbit) or as accessories develop the metabolism may gradually rise to normal. In these animals the average maximum reduction in metabolism is around 35 per cent to 40 per cent, which corresponds closely to that observed in the severest forms of human cretinism and myxedema and may be designated as the myxedema level.

While qualitatively the symptoms following thyroidectomy in both young and adult animals are similar, the visible manifestations are strikingly more prominent in animals thyroidectomized during the period of growth. Adult herbivora like the rabbit, sheep or goat may show very little change clinically beyond a dryness and thickening in the skin, thinning of the hair, a gain in weight and a lowering of body temperature. This was observed by the earlier workers. Heat production measurements in these animals, however, show the usual marked decrease. In the young there are in addition the gross manifestations of stunted physical, mental and sexual development.

With a knowledge of the frequency and functional importance of accessory and aberrant thyroid tissue, the writer believes that in the adult animal (sheep, rabbit, cat) the thyroid is not essential for vegetative life, while in the young it is only indirectly essential in that it is necessary for growth and development. The thyroid is an organ acquired late in the development of animal life, present only in the higher Chordates, and all we know of its function indicates that it provides the means for maintaining a higher level of metabolism and for varying its rate.

b. Biochemistry. The next most important advance in thyroid physiology was the demonstration in 1891 by Murray (82a), of the remarkable therapeutic effect of the injection of a glycerol extract of fresh sheep's thyroid in cases of Gull's disease. This was quickly followed by the independent announcements in 1892 by Howitz (83), by Mackenzie (84) and by Fox (85), that thyroid either fresh or dried or boiled was equally efficacious when fed by mouth. Emminghaus and Reinhold (86) in 1894 showed that thyroid feeding also caused a marked reduction in the size of goiter. These discoveries of the therapeutic effects of thyroid feeding also mark the beginning of thyroid biochemistry. The names of Hutchinson (87), (88), (89) in Great Britain, Frankel (90) in Austria, Baumann, Roos (91), Oswald (92) and Dréchsel (93) are most closely associated with the biochemical work of this early period which culminated in the announcement in 1895 by Baumann (94), (95), (96) of Freiburg that iodin in a rather firm organic combination was a normal constituent of the mammalian thyroid. He obtained a substance by acid hydrolysis which he named "iodothyrine." This substance was later put on the market by a pharmaceutical firm under the name of "thyroidin." The substance obtained by Baumann was a brown amorphous powder insoluble in water and acids, slightly soluble in alkalies, gave no protein reactions, contained 0.4 to 0.5 per cent P. and as high as 9.3 per cent iodin. Later work has shown that this method of hydrolysis (10 per cent H_2SO_4) partially destroys the specific iodin compound. Baumann and his pupils, Roos and Goldman (97), showed that iodin was present in the mammalian thyroid in very variable amounts and that feeding iodin increased the store. Oswald (98), (99), in 1899, observed that the iodin was contained in the colloid, and that the colloid of the thyroid was mainly globulin, and introduced the terms thyreoglobulin and iodothyreoglobulin. He showed that the iodin content varied in general with the amount of visible colloid in the glands, but that hyperplastic glands could be rich in globulin and iodin-free. The relation of iodin to the structure of the thyroid has been particularly studied by Marine and Williams (100) and Marine and Lenhart (101). These studies have cleared up the controversy that had developed concerning the fundamental importance of iodin in thyroid physiology, because many workers noting the absence of iodin in the thyroid in certain conditions still claimed it was only an accidental constituent probably excreted into the thyroid as a waste product. Much of the earlier work claiming the absence of iodin in the thyroid was, of course, due to faulty methods of chemical analysis. The final results in all of this work showed that the iodin store in the thyroid varies in general with the amount of stainable colloid, inversely with the degree of active hyperplasia and in the extreme degrees of active hyperplasia seen in cretinoid states in man and animals the iodin store may be entirely exhausted. In the following table the relation of iodin to histological structure as found by Marine and Lenhart are given:

	NORMAL	EARLY HYPERPLASTIC STAGE	MODERATE HYPERPLASTIC STAGE	MARKED HYPERPLASTIC STAGE	COLLOID OR RESTING STAGE
Man............	2.17*	0.88	0.71	0.32	2.00
Dog............	3.32	0.62	0.37	0.11	1.99
Sheep..........	2.47		0.40	0.01	3.00
Ox.............	3.46	1.65		0.19	
Pig............	2.51	1.10			2.35

* Iodin in milligrams per gram dried gland.

Earlier workers reported discordant results regarding the presence of iodin in the fetal thyroid (102). Fenger (103), (104), using standard methods of analysis, showed conclusively in a large series of animals that iodin is present in the fetal thyroid of cattle, pigs, sheep and man. In cattle it is present as early as the third month of intra-uterine life, that is, six months before birth. The iodin content gradually rises with the increasing age of the fetus and shows the same variations dependent upon structure as seen in extra-uterine life. The writer has made similar observations on a large series of fetal thyroids of dogs. The iodin store in the thyroid shows striking seasonal variations, being highest in the early autumn (October) and lowest in the early spring (April) in this latitude (Seidell) (105), (106). The thyroid has an extraordinary affinity for iodin (107), (108). This was first observed by Baumann in 1895, though iodin has been knowingly used in the treatment of goiter since 1820 (Coindet) and unknowingly throughout many parts of the world, civilized and uncivilized, for unknown centuries. Iodin given to pregnant mothers is also readily stored in the fetal thyroid. The amount of iodin taken up by a given thyroid varies with the degree of active thyroid hyperplasia. The maximum store per gram being relatively constant, for most mammals thus far examined averaging between 5 and 5.5 mgm. per gram of dried thyroid (109). The minimum amount necessary for the maintenance of normal gland structure is likewise relatively constant, averaging about 1 mgm. per gram of dried substance (110). The average normal iodin content for human thyroid is around 2 mgm. per gram of dried substance and the maximum total store of iodin in a strictly normal human thyroid does not exceed 25 mgm. (111), (112), (113), (114). These facts are of the utmost importance in the practical application of iodin to the prevention of goiter.

Perfused surviving thyroids show the same marked ability to take out and store iodin from the circulating fluid as is seen in the thyroid in situ (115), (116), (122). It has been shown that the iodin content of the thyroid of a dog may be increased several hundred per cent within five minutes after the injection of 50 mgm. of potassium iodid into the femoral vein. As much as 18.5 per cent of a single intake of 38 mgm. (50 mgm. KI) given to a dog by mouth may be recovered from the thyroid whose ratio to body weight was as 1:687. The thyroid stands alone at present among the specific affinities of tissues for inorganic substances.

The older literature contains many reports of the presence of appreciable amounts of iodin in the parathyroids, thymus, pituitary and other organs. Excluding its presence in tissue due to the recent administration or to contact with iodin, active normal tissues other than the thyroid do not contain amounts greater than could be accounted for by its discharge from the thyroid. A great deal of work has been done in the attempt both to isolate and to synthesize the active iodin compound of the thyroid, especially by Oswald (117), Nürnberg (118), Koch (119) and many others (120), (121). Whole proteins—casein, glutein, serum-albumin and globulin and many amino acids including tyrosin, tryptophane, histidin and phenylanilin have been iodized but none of these were active. Later work, using the tadpole as a test object, showed that iodized blood serum, especially the globulin fraction, has a slight thyroid-like effect (123). In 1916 Kendall (124) reported the isolation of the specific iodin compound in crystalline form which he named "thyroxin." According to his latest report (125) this substance has the empirical formula $C_{11}H_{10}O_3NI_3$ and structurally is trihydro-triiodo-oxy-beta indoleproprionic acid. This substance in the purest form yet obtained contains 65 per cent iodin, has a melting point of around 250 and crystallizes in sheaves of needles. Kendall has shown that this substance produces the same pharmacological effects as whole thyroid. Kendall also showed that iodin is present in the thyroid in both an active and inactive form (126). Taking advantage of the extraordinary affinity of the thyroid for iodin and of the Gudernatsch tadpole test, Marine and Rogoff (127) carried out experiments to determine the rapidity of the production of active thyroid iodin. Differences in pharmacological activity of the thyroid were detectable in eight hours after intravenous injection of 50 mgm. KI and the differences in activity were quite marked after thirty hours. These observations indicate that while the storage of iodin is instantaneous the formation of thyroxin is a relatively slow process.

In 1895 Magnus-Levy (128), (129) reported in Gull's disease that the respiratory exchange was markedly decreased below normal and that in Graves' disease it was notably increased. He also demonstrated that feeding thyroid to cases of Gull's disease markedly increased their respiratory exchange and the excretion of urinary nitrogen. Friedreich Müller had recognized the increased nitrogen excretion in Graves' disease in 1893 (130). These discoveries by Magnus-Levy are the most important contributions to the pharmacology of thyroid substance and among the most important contributions to our knowledge of thyroid physiology. The most characteristic pharmacological action of thyroid or of its isolated active substance, thyroxin, is an increase in total metabolism (131), (132), (133), (134), (135), (136), (137). Its action is in general proportional to its iodin content as determined either by measurements of heat production, nitrogen excretion or the Gudernatsch tadpole test (138) (the most sensitive test yet developed for thyroid). In tadpoles thyroid substance causes a rapid loss in weight associated with metamorphosis in a few days. This is proportional to the active iodin (139). Some have considered that the action of thyroid on tadpoles was a specific stimulus to differentiation. All the phenomena observed may be explained on the well-known action of the thyroid in accelerating metabolism and the apparent specificity does not depend on a new specific action of thyroid but on its application to a living organism at a specific period in its development (140). The acetonitrile test of Hunt and Seidell (141), (142), (143) is not specific for thyroid activity, since while thyroid feeding increases the resistance of white mice to acetonitrile poisoning it decreases it in rats and guinea pigs. Further, removal of the thyroid does not alter the response in mice and the blood of thyroidectomized animals also protects. In man, Plummer (144) has roughly estimated that for the normal individual approximately 1 mgm. of thyroxin daily is sufficient for normal metabolic activity.

The effect of thyroid on the heart and circulation has been particularly studied by von Fürth (145), (146), von Cyon (53) and Oswald (147). Aqueous extracts of the whole gland injected intravenously caused the usual lowering of blood pressure, while purified solution of iodothyreoglobulin causes only a slight lowering of blood pressure but the heart rate is notably increased after a latent period. Oswald believes that thyroid increases the irritability of all sympathetic nerve endings. The thyroid has no specific effect on blood coagulation. In Graves' disease the coagulation time is usually prolonged but attempts to establish a thyroid relation have been negative (148), (149), (150). The relation of the thyroid to immunity has received a great deal of attention and the literature is confusing and contradictory. In general it has been found that hemolysin and agglutinin formation are higher in thyroidectomized than in control rabbits, while antitoxin (diphtheria) formation is lower in thyroidectomized animals (dog, horse and rabbit) (151), (152), (153), (154), (158), (159), (160). Fjeldstadt (155) in eighteen thyroidectomized rabbits found no increase in agglutinin formation. Most observers have reported numerous exceptions to the above general statement except Ecker and Goldblatt (156) who found the hemolytic titer of thyroidectomized rabbits always higher than the controls. It is stated that anaphylactic shock does not occur in guinea pigs if sensitized after thyroidectomy but does occur if sensitized before thyroidectomy (157). At present the results obtained do not warrant any direct association of the thyroid with antibody formation. The reaction to infections as shown by a reduction in iodin store and a tendency to hypertrophy and hyperplasia clearly indicate the thyroid is an important indirect factor in resistance to infections. The increased heat production in infections is to some extent dependent upon the thyroid.

c. Regeneration and transplantation. The mammalian and avian thyroid regenerates rapidly after partial removal. Two major factors—the amount removed and the administration or the withholding of iodin, and probably many minor factors—age, diet, species, determine the degree of regeneration (70). In the dog, if one removes three-fourths of the gland, ordinarily regeneration occurs in the remaining fourth, but if small amounts of iodin are given, such regeneration does not take place. If as much as nine-tenths of the gland is removed, iodin in any amount does not protect against regeneration. Halsted (68) made an extensive study of thyroid regeneration in 1889. Ribbert (69) showed that regeneration may begin within a few days after partial removal and occurs first in the sub-capsular zone. His suggestion that this centrifugal growth is dependent upon a more active blood supply is probably correct. The irregular insular hyperthrophy and hyperplasia often seen in human goiter may be thus explained. Anatomically and chemically the thyroid changes in regeneration are identical with those occurring in the spontaneous hyperplasias of simple goiter and are controllable by the same methods, i.e., cellular hypertrophy and hyperplasia do not occur until after the iodin store falls below a given level (0.1 mgm. per gm. dried) (70).

Transplantation of the thyroid has been extensively studied by Cristiani (71), L. Loeb (72) and his co-workers, and by Manley and Marine (73). Thyroid tissue autografts readily in any part of the body and shows all the chemical and morphological reactions seen in the non-transplanted tissue. Growth of the transplant varies inversely with the degree of thyroid insufficiency created in the host. The administration of iodin or desiccated thyroid inhibits the growth of thyroid transplants. In much of the older work on transplantation, attempts to transplant large pieces, even whole glands, were failures. As only the peripheral layer of not more than four to six cells in thickness survive, the ideal transplant is a slice of tissue about 50 microns in thickness laid on some flat surface, as the subcutaneous tissue or the sheaths of muscles. The frozen thyroid tissue of rabbits also transplants readily (Manley and Marine). Many such experiments were made where the tissue was frozen with carbon dioxide from one to five minutes. The temperatures reached were not measured. Homeografts are rarely permanent. Barring technical errors, they all "take" but begin to undergo absorption as early as the seventh or eighth day.

Some animals destroy initial homeografts much more slowly than this, indicating that there are different degrees of foreignness of the transplanted proteins in animals as well as in man. In man, by transplanting within the same blood group it is probable that the average life of homeografts might be somewhat prolonged. But there are such great differences within a given group which cannot be detected by the usual hemolysin or agglutinin tests, that permanent value from homeotransplantation is at present hopeless and must continue to be until some means is discovered to overcome the foreign protein reaction to the grafted tissue.

Heterotransplantation of thyroid in mammals never succeeds.

d. Diet. Diet notably affects both the structure and chemistry (166), (167), (168), (169). Baumann (96) in 1896 and many others (161) noted in dogs that fresh meats caused hypertrophy of the thyroid, while sea fish (cod) increased the iodin store. Watson (162) also found that a meat diet caused hypertrophy and hyperplasia of the thyroid cells in rats. Marine and Lenhart (163) (164), (165) showed that liver, particularly pigs' liver, was the most potent of a great variety of meats in causing thyroid hyperplasia in dogs and cats and also this food was an important factor in the causation of goiter in brook trout. Recent work by McCarrison (170), confirmed by Mellanby (171) showed that fats were even more potent in producing thyroid hyperplasia. McCarrison's suggestion that his effect is in part dependent on an increased bacterial putrefaction in the intestine seems unlikely. As thyroid hyperplasia is secondary to the depletion of the iodin store, these facts indicate that diets rich in proteins and fat increase the rate of discharge of iodin. It seems probable that thyroid activity is more necessary for the oxidation of fats and of proteins than of carbohydrates. Carbohydrate diets do not cause thyroid hypertrophy, as has been shown by McCarrison. Inanition brings about involution of the thyroid, decrease in the size of the epithelial cells and increase in colloid (172), evidence of decreased functional activity.

INTERRELATIONS. We are only at the beginning of definite knowledge concerning its functional interrelations with other tissues. During the last decade this subject has become involved in a stupendous mass of ill-advised speculation, exploitation and fiction. Interrelations may be either inhibitory (antagonistic) or augmentory in nature. Sufficient facts are available to indicate that these correlations determine the thyroid's most important effects on nutrition. These effects are brought about by acceleration and inhibition of its functional activity which in turn are caused by chemical factors, positive or negative, acting through the blood stream either directly on the gland cells or indirectly through nerve impulses. Further, we must recognize that inhibition and acceleration of tissue activity may be brought about by the presence of a specific hormone or by its absence and that apparent acceleration may be an actual loss of inhibition and vice versa.

Thyroid-Parasex glands. (*Suprarenal cortex, interstitial and luteal cells.*) The relation of the thyroid to the sex glands was known to the ancients in its crudest external manifestations—the thyroid enlargement with menstruation, puberty, pregnancy and menopause (173). This relationship has passed down to our time with no proved additions to our knowledge concerning it (174). Recently it has been demonstrated that when the suprarenal cortex in rabbits is sufficiently injured as by freezing or by partial removal, a marked chronic increase in heat production usually occurs (80 per cent in a series by Marine and Baumann) (175). This increase usually begins within three to six days after the suprarenal injury and may last from two weeks to several months. Heat production may be increased to 60 per cent or more above the normal. On the other hand if the thyroid gland is removed and the metabolism allowed to fall to the myxedema level prior to the injury to the suprarenal cortex, this increase in heat production does not occur (82). Scott (176) has confirmed these findings, using cats. Golyakowski (177) in 1899 in a brief preliminary report observed increased CO_2 output in dogs following ligation of the suprarenal vessels. There is some evidence that the increased heat production is associated with a loss of iodin from the thyroid and recently Black, Hupper and Rogers (178) have published evidence that feeding "suprarenal gland residue" to dogs increased the iodin store of the thyroid. This reaction with increased heat production appears then to be a suprarenal cortex thyroid interrelationship. Our present interpretation is that the suprarenal cortex exercises a regulatory or inhibitory control over thyroid activity and when this is withdrawn the thyroid automatically responds with increased function. It should be pointed out that there is evidence that the suprarenal cortex exercises an inhibitory control over other tissue functions as well—the thyroid suprarenal interrelation, therefore, is not an isolated one. The practical application of these observations may be of great importance; for example the enlargement of the thyroid at puberty, during menstruation, pregnancy and menopause may be thus partly explained. The effect of bacterial toxins in causing thyroid hyperplasia may be in part determined by a primary injury to the suprarenal cortex. Other well-known facts involving obvious interrelations as, for example, the hypersusceptibility of certain individuals to adrenalin or the hypersusceptibility of certain individuals to desiccated thyroid and thyroxin, probably have as their basis this fundamental thyroid suprarenal cortex interrelationship. Exophthalmic goiter is in some way intimately involved in this interrelation and the popular conception that this disease is a primary thyroid disease must give way to a broader conception in which cortical exhaustion indirectly, and epinephrin stimulation directly, are in my opinion important primary factors in bringing about increased thyroid activity. Enlargement of the suprarenal cortex with an inrease in the epinephrin store has been observed following prolonged feeding with desiccated thyroid (179), (180), (181), (182), (183). During starvation, enlargement of the suprarenal cortex has been observed. Both of these phenomena may be interpreted as an attempt to suppress thyroid activity.

Finally, the normal involution of the suprarenal cortex in infants should be mentioned. This remarkable destruction of the reticular and fascicular zones of the cortex has been observed only in infants and begins during the second or third week of extra-uterine life (183a). The process is initiated as a hemorrhagic infiltration of the two inner zones and goes on to necrosis, destruction and absorption of these layers with collapse and folding of the glomerular zone on to the medulla. The duration of the stages of absorption and healing is indefinite. Some authors estimate it at two to three weeks and other at two to three months. The end result, however, of this destruction is a marked decrease in the volume of cortex so that a child one year old has a smaller total volume of cortex than at birth.

The physiological significance of this rapidly progressive partial destruction of the cortex is unknown. It is not accidential or traumatic. Its occurrence in accessory suprarenals as well suggests that it is a systemic purposeful reaction to meet the altered conditions incident to extra-uterine life. In the light of the relation of experimental injury of the suprarenal cortex in rabbits, dogs and cats to increased heat production, it is suggested that one of the effects of the cortical destruction in infants may be increased heat production through thyroid activation. All that can be said at present is that the cortical injury and increased heat production in infants begin at approximately the same time and parallel each other.

Thyroid-Chromophil tissue. Epinephrin injected intravenously causes a marked constriction of the thyroid vesssels. An interrelationship of function was first postulated by Eppinger, Falta and Rudinger (184). They assumed that the chromophil system directly stimulated the thyroid. This conception received experimental support from the work of Asher and his pupils (186), who in 1910 showed that the blood pressure response in rabbits to a given dose of adrenalin was greater after stimulation of the thyroid nerves with intact thyroid than before such stimulation. This has been confirmed from several sources and especially by Cannon and his co-workers. The Goetsch epinephrin test in exophthalmic goiter is a clinical application of this reaction. Oswald has shown that a similar increase in the reaction to epinephrin may be obtained by injecting iodothyreoglobulin instead of stimulation of the thyroid nerves. The nature of this reaction is still in doubt (187), (188). Asher and Flack believe that the thyroid hormone increases the irritability of or sensitizes the tissues innervated by the sympathetic nervous system in some way so that it is more susceptible to stimulation by epinephrin.

Thyroid-Gonad interrelationship. Very little is known (185). That there is an important direct relation between some constituent of the gonads, especially in the female, and the thyroid is certain. The exceedingly complex nature of the sex gland has thus far been a perfect barrier to trustworthy experimental investigation. Total removal of the gonads usually leads to a slight depression of the thyroid function (189).

Thyroid-Hypophysis. Rogowitsch (190) and others (191) (192), (195), (196) have reported marked enlargements of the anterior lobe and especially the pars intermedia following thyroidectomy—as much as 400 per cent. They interpreted this enlargement as indicating that the pituitary could function vicariously for the thyroid. Subsequent work by Simpson and Hunter (193) and by the author has not confirmed this. In rabbits there is a slight hypertrophy of the anterior lobe, but this is rarely more than 15 to 20 per cent after five or six months. Many investigators have found traces of iodin in both the human and sheep hypophysis—others have failed to find it. Simpson and Hunter (194) showed conclusively that the sheep pituitary contained no iodin even in animals recently fed with this element. Livingston (197) on the other hand has published a few observations indicating that desiccated thyroid protects male thyroidectomized rabbits against pituitary hypertrophy. No pituitary hypertrophy was observed in thyroidectomized female rabbits even without thyroid feeding. Hewitt (198), however, reported that thyroid feeding in white rats caused pituitary enlargement. The idea that the thyroid and pituitary were functionally related seems to have been suggested by Virchow and was based on the morphological resemblance of the colloid filled follicles of the para intermedia to the thyroid follicle. Acromegaly is usually associated with a slight increase in the size of the thyroid and with an increase in heat production rarely over 20 per cent. None of the facts thus far established suggest any direct functional relation between the hypophysis and the thyroid.

Thyroid-Thymus. There is no evidence of any important relation between these two organs despite a relatively large literature dealing with their functional interdependencies (199). There is no doubt that the thymus is usually enlarged or persistent in many conditions in which the thyroid is involved, for example, simple goiter, myxedema, Graves' disease. Asher and Ruchti (200) found no change in the respiratory exchange after thymectomy in rabbits whether performed before or after thyroidectomy. Gudernatsch thought thymus feeding inhibited to some extent the action of the thyroid feeding on tadpoles. Baumann (unpublished) has found that foods enriched by the addition of protein-free nucleic acids of any origin stimulate growth in tadpoles. That there is an important indirect relation between the thyroid and thymus through the sex glands and suprarenals is certain since each of these glands is closely associated functionally with the sex and parasex tissues, and both the thyroid and the thymus are usually affected in conditions involving the suprarenals or sex glands, as for example, Addison's disease, status lymphaticus, castration, Graves' disease, etc.

Thyroid-Spleen. Asher and his pupils (201) have recently revived the question of the thyroid-spleen interrelationship suggested by Tauber (202) in 1884. Asher found that splenectomized rats with intact thyroids were less resistant to reduced oxygen pressures than normal rats, or rats with combined thyroidectomy and splenectomy. Splenectomy has been found to slightly increase the respiratory exchange in rabbits but this also occurs when the thyroid is removed.

Thyroid-Pancreas and Liver. Falta thought the thyroid and pancreas were antagonistic (203). He stated that an epinephrin injection which in normal dogs caused a marked glycosuria does not produce glycosuria in thyroidectomized dogs. Similar observations were made by Grey and de Sautelle (204) on dogs and by Pick and Pineles on goats. Underhill (205), however, denies that thyroidectomized dogs in which great care has been exercised to preserve the two external parathyroids are less susceptible to adrenalin glycosuria. Thyroid feeding was found to produce a marked decrease in the diastatic activity of the pancrease of white rats and this was often associated with enlargement of the pancreas (206). Clinicians have reported the frequent association of some of the symptoms of Graves' disease with acute pancreatitis (207). It has also been suggested that the lowered sugar tolerance and glycosuria of Graves' disease might involve a thyroid-pancreas interrelation. The increased alimentary tolerance for glucose in myxedema or after thyroidectomy is of doubtful significance. It may be due to decreased absorption from the intestine rather than to a direct thyroidectomy influence. What evidence there is seems to indicate that any thyroid-pancreas interrelationship is an indirect one and dependent on epinephrin sensitization. Whipple and Christman (208) have shown that thyroidectomy does not influence the excretion of phenoltetrachlorphthalein by the liver.

Thyroid-Parathyroids. No interrelation of function is known. The earlier affirmative statements were based on faulty methods and errors in interpretation.

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INTRACELLULAR DIGESTION

The Enzymes and Anti-Enzymes Concerned

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All of the protozoa and many somewhat higher animals obtain their food by intracellular digestion. Among the lower metazoa including sponges, coelenterates and some of the lowest forms of worms, such as turbellaria, cells lining the digestive cavity act as phagocytes, ingest solid particles of food and dissolve them usually within vacuoles in their cytoplasm. Among the coelenterates and turbellaria food undergoes preparatory changes within the enteric cavity but the part of enzymes poured out into the cavity is doubtful. Extracellular digestion by enzymes secreted into a digestive tract is first definitely established in the ascending animal scale in the higher worms, such as nematodes, earth worms, etc., and becomes the sole method for the digestion of food.[1]

A proteolyte enzyme has been extracted from the bodies of amoebae and from the phagocytic cells of coelenterates, such as the sea anemones; it digests protein in a weakly acid and in a weakly alkaline medium. When the amoeba or the digesting phagocytes of higher forms ingest particles of blue litmus they are quickly turned to red but after a time the vacuole surrounding the granule loses its free acid and the indicator shows a neutral or alkaline reaction and many other indicators exhibit similar reactions.

In the lowest metazoa, namely, in the sponges, and in all higher forms which he examined, Metschnikoff (67) found mesoblastic phagocytes capable of approaching and ingesting foreign material which has found its way into the tissues of the organism. Among mammals the cells which exhibit these properties are notably the polynuclear leucocytes of the bone marrow and blood and certain mononuclear wandering cells which are widely scattered in the tissues. These cells approach or fix foreign particulate matter which has entered the body, engulf it and so far as they are capable bring about the solution of the ingested material. Not only is foreign material subject to the activities of these cells but body substance, including cells and intercellular substance, which has been injured or killed, may undergo some change as the result of which it becomes the prey of the phagocytic cells and is eliminated by intracellular digestion.

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[1] The literature of phagocytic digestion of food in lower animals has been reviewed by O. von Fürth, *Vergleichende chemische Physiologie der niederen Tiere*, Jena, 1903 and by H. Jordan, *Vergleichende Physiologie wirbelloser Tiere*, Jena, 1913.

When phagocytic cells of the higher animals are allowed to ingest indicators, such as litmus, tournesol-blue or alazarin sulphate, no change occurs. With neutral-red, bacteria and the nuclei of cellular elements ingested both by polynuclear leucocytes and by macrophages assume a brownish red color which Metschnikoff has attributed to a feebly acid reaction.

ENZYME OF THE POLYNUCLEAR LEUCOCYTES; LEUCOPROTEASE. The presence of peptone in pus was observed by Eichwald (16) in 1864, and later this observation was confirmed by Maixner (66) and by Hofmeister (31). In association with diseases characterized by pus formation, namely, empyema, purulent peritonitis, cerebro-spinal meningitis, etc., Maixner found peptone in the urine. The occurrence of proteolytic enzymes in the cells of purulent exudates was first demonstrated by Friedrich Müller (76), who showed that a glycerine extract of purulent sputum digests fibrin or coagulated protein in the presence of a weakly alkaline reaction. Similar enzyme was demonstrable in fresh pus from an acute abscess but was absent in the thin fluid from a "cold abscess." Leber (60) and later Achalme (1) showed that pus liquefied gelatin, and dissolved fibrin, egg albumin coagulated by heat, and casein.

Methods. Many methods used for the study of pepsin and trypsin are not applicable to the weaker enzymes present in phagocytic cells. Fresh fibrin frequently employed is not well adapted to the study because it contains enzymes which bring about autolysis. Some very simple methods have been introduced with the hope that they would be useful to physicians and numerous clinical studies have been made with their aid. The methods which have been most used will be cited briefly.

Liquefaction of gelatin: This method introduced by Fermi (21) has been used by Eppenstein (18) for the demonstration of enzymes in leucemic blood. Gelatin in the strength of 6 to 8 per cent is used as a substrate for the demonstration of enzymes. The substance to be examined is mixed in quantity of 0.2 to 0.5 cc. with 1 to 2 cc. of gelatin containing 1 per cent soda and kept in an incubator at 37° to 40°C. during 12 or more hours. Should digestion occur the gelatin remains liquid after cooling with ice during several hours.

Digestion of casein: Gross (25) and Fuld (23) have introduced a method for the study of tryptic digestion which has been much employed in the study of the anti-enzymotic activity of the blood serum. Casein in 1 per cent solution in $\frac{1}{10}$ N sodium hydroxide is neutralized with $\frac{1}{10}$ N hydrochloric acid and diluted with five times its volume of physiological salt solution. After the enzyme has acted upon this substrate the mixture is acidified by 5 per cent acetic acid in weak alcohol and if digestion is complete the solution remains clear, since the digestion products of casein are not precipitated in acid solution, but if undigested casein remains it is precipitated. Numerous studies of the anti-enzymotic activity of the blood serum with carcinoma and other disease have been made by this method. The amount of serum necessary to inhibit a given quantity of enzyme is determined.

Serum plate method: Eduard Müller and Jochmann (71) have used, for demonstration of proteolytic enzymes, Loeffler's medium spread out as solid plates in Petri dishes. The medium consists of two parts of beef serum and one part of bouillon containing approximately 1 per cent of glucose and coagulated by exposure to a temperature from 85 to 95°C. during several hours. A drop of the material under investigation is placed upon the surface of the plate which is then incubated at from 55° to 60°C. during 24 hours. At this temperature a shallow cup-like depression is formed by the action of the enzyme upon the coagulated beef serum. Excavation of the surface of the plate does not occur at body temperature and the higher temperature has the advantage that it prevents the multiplication of bacteria. Various dilutions of the fluid to be tested for enzymotic activity may be prepared and the titer at which digestion ceases may be determined. Many drops may be applied to the same plate. This somewhat crude method has been widely employed for the study of enzymes and anti-enzymes both in health and disease but it has in great part served to confirm observations made by more accurate quantitative methods. The observations made upon leucocytic enzymes and anti-enzymes by this method have been fully reviewed by Weins (102) but he has overlooked almost all of the observations made by quantitative chemical methods.

Measurement of digestion by nitrogen determination: When the enzyme digests a protein substrate the activity of digestion is measured by the amount of protein coagulable by heat or by chemical means which has been split into incoagulable products such as albumose, peptone and lower nitrogen-containing decomposition products. This measurement is readily made by determining the amount of nitrogen in incoagulable substances before and after digestion during 24 hours at 37°C., some conveniently obtainable protein such as blood serum denaturalized by heat being used as a substrate. Nitrogen may be determined by the Kjeldahl method which was used by Ascoli and Maresche (4) and by Opie (79), or by the microchemical method of Folin, which Jobling (49) used. The method ensures an accurate determination of the activity of digestion.

Preparation and character of leucoprotease. The enzyme of the polynuclear leucocytes was extracted (Friedrich Müller) from purulent sputum or fresh pus by means of glycerine. A permanent preparation of the enzyme may be obtained from the leucocytes of a sterile inflammatory exudate produced by the injection of aleuronat into the pleural cavity of a dog by treating the washed cells with absolute alcohol in sufficient quantity to cause dehydration (Opie). After removal of the alcohol the cells are treated with ether, dried and reduced to a fine powder. This material actively digests protein such as blood serum denaturalized after dilution with an equal volume of physiological salt solution by heating to 75°C. It acts in a neutral or alkaline (0.2 per cent sodium carbonate) solution but is almost wholly inactive in the presence of acid (0.2 per cent acetic acid). This enzyme is slightly increased in activity by short exposure to temperatures between 50 and 60°C., is slightly impaired by a temperature of 65°C. and is destroyed between 70 and 75°C.

Fresh leucocytes incubated on Loeffler's serum plates at 37°C. cause no cupping of the surface but if leucocytes are first subjected to an elevated temperature (55°C.) and then incubated at body temperature active solution of the plate occurs. The high temperature according to Müller and Jochmann is required to destroy the leucocytes so that their enzymes may be set free. The enzyme causes active proteolysis at temperature between 50° and 55°C.

The proteolytic enzyme of the polynuclear leucocytes has been given the name "leucoprotease" by Opie (81).

A purified enzyme was prepared from leucocytes by Jochmann and Lockemann (57). Material containing the enzyme was allowed to autolyze during from 24 to 48 hours at 55°C. The autolysate was then treated with about five times its volume of alcohol (2 parts) and ether

(1 part) in order to remove fatty material and to precipitate protein. After standing during one day, the material was filtered and the residue, first evaporated to get rid of alcohol and ether, was intimately rubbed with glycerine in the proportion of about one-fourth of the volume of the original material from which the enzyme was obtained, and an equal volume of water was added. After standing one or two days in the dark the solution was passed through a porcelain filter and the clear filtrate was treated with five or six times its volume of a mixture of alcohol and ether. The white precipitate thus formed was dried in a vacuum over concentrated sulphuric acid. The final product which was yellowish brown and somewhat hydroscopic formed a brown solution when dissolved by rubbing with water or physiological salt solution. It digests coagulated blood serum, fibrin, gelatin and casein best in a weakly alkaline solution. When dissolved in water it is destroyed by temperatures between 70° and 75°C. but in dry form though impaired in activity resists temperatures from 75° to 95°C. being destroyed by 100°C.

The effects of various chemicals upon the enzyme of leucocytes have been studied by several observers, including Jochmann and Lockemann (57), Müller and Kolaczek (75). The enzyme is especially resistant to the action of formalin. Jochmann and Ziegler (58) noted that the proteolytic enzyme of leucemic organs was not destroyed by preservation in 10 per cent formalin even after the lapse of a year. If part of a spleen is placed in salt solution and part in 10 per cent formalin (Jochmann and Lockemann) disintegration proceeds rapidly in the former at a temperature of 55°C. so that complete liquefaction has occurred after 48 hours, but in the presence of formalin the tissue is completely preserved. If tissue preserved in formalin is washed in running water and then applied to the serum plate, excavation of the surface occurs. The experiment shows that digestion is inhibited by the presence of formalin but the enzyme is not destroyed. The purified enzyme of Jochmann and Lockemann was impaired but not destroyed when dissolved in 10 per cent formalin. It is noteworthy that the action of formalin on trypsin is similar to that upon the enzyme of leucocytes.

The production of fever by leucoprotease was observed by Jochmann (54). He found elevation of temperature in rabbits following the injection of enzyme of leucocytes or of pancreatin into the vein or into the subcutaneous tissue; it appeared after one hour, the temperature remained somewhat elevated after two hours and then fell. The result was the same when the solution was heated during a quarter of an hour at 80° to 90°C. and is not dependent upon the presence of the proteolytic enzyme but is caused by substances related to proteins and associated with the enzyme. Jochmann thinks that these substances, as well as the toxins of bacteria, are concerned in the production of the fever which accompanies suppurative processes.

Diminution of the coagulability of drawn blood by addition of enzyme of leucocytes has been observed by Jochmann (54). Very large quantities of the enzyme injected into an animal cause an initial diminution of coagulability and later an acceleration of coagulation.

Occurrence of leucoprotease in the tissues. The proteolytic enzyme of polynuclear leucocytes which like trypsin digests in the presence of an alkaline reaction has been found in those organs within which polynuclear leucocytes are particularly numerous. Leucoprotease was found in the bone marrow by Opie (80). Autolysis of liver, kidney, spleen lymph nodes and other tissues proceeds more rapidly in the presence of weak acid (e.g., 0.2 per cent acetic acid) than in a neutral or alkaline medium. Bone marrow, on the contrary, autolyzes more actively in an alkaline (e.g., 0.2 per cent sodium carbonate) than in an acid medium and furthermore digests extraneous protein under the same conditions. E. Müller and Jochmann (71) found that a drop of the material which was pressed from the cut surface of a human lymph node failed to digest the surface of coagulated serum incubated at 50°C. but under the same conditions active digestion was caused by red marrow squeezed out of a rib or vertebra.

Leucoprotease is formed within the polynuclear leucocytes before they leave the bone marrow. Here they elaborate a proteolytic enzyme which in some respects resembles trypsin. Nothing is known concerning the formation of the enzyme with the leucocyte. A zymogen has not been found. No relation to the specific granules of the polynuclear leucocytes has been demonstrated.

Hedin and Rowland (28) found that the expressed juice of the spleen of beef, horse, pig and sheep undergoes much more active autolysis in an acid than in an alkaline medium. Nevertheless in the presence of an alkaline reaction (0.2 to 0.37 per cent sodium bicarbonate) digestion is still considerable. Hedin (26) succeeded in separating almost completely two enzymes one of which, designated lieno-B-protease, digests in an alkaline medium. These enzymes not only digest the cells of the spleen but cause the disintegration of other proteins such as fibrin, casein and coagulated blood serum.

By quantitative determinations by means of the Kjeldahl method, Opie (80) found that the digestive activity of spleen in the presence of an alkaline medium stood next to that of bone marrow and exceeded that of liver, kidney and lymphatic nodes.

The proteolytic enzyme of leucocytes has been found by Friederich Müller and subsequent investigators in purulent exudates from patients. The studies of Opie were in great part made with the active leucoprotease obtained from sterile inflammatory exudates of dogs. E. Müller and Jochmann (72) using the serum plate method, have found no solution of the serum with cells obtained from rabbits and guinea pigs by injecting a solution of nucleinic acid below the skin or into the peritoneal cavity. Using the same method, Jochmann and K. Ziegler (58) have observed proteolysis caused by spleen and bone marrow of monkeys and slight proteolysis caused by the same tissues from the dog but have found none when they tested organs from fox, cat, various rodents, pig, sheep, beef and horse. The serum plate method is not sufficiently delicate to determine the absence of enzyme. A weak proteolytic enzyme digesting in the presence of an alkaline medium was found by Opie and Barker (84) in leucocytes obtained by injecting turpentine into the subcutaneous tissue of a rabbit; digestion was measured by Kjeldahl determination of nitrogen in the products of digestion. Using Van Slyke's micro-method to measure amino nitrogen, Parker and Franke (87) found only a very small increase after digestion of purified blood albumin and came to the conclusion that leucocytes of rabbits contain no proteolytic enzyme; they found, however, erepsin, which formed amino acids by digestion of peptone. No enzyme resembling the leucoprotease of mammals was found by Opie and Barker (84) in the leucocytes, bone marrow or spleen of the hen.

Relation of leucoprotease to trypsin. Leucoprotease which acts in the presence of an alkaline medium resembles trypsin but extracts obtained from leucocytes are much less active than preparations of trypsin. Those who have assumed that the anti-enzyme of the serum is a true antibody have reached the conclusion that leucoprotease and trypsin are identical for as Jochmann and Kantorowicz (56) have found, injection of animals with one of the two substances increases the inhibiting action of the serum for both. Furthermore, serum of which the inhibiting action is overcome by addition of one enzyme no longer inhibits the other. This evidence is inconclusive if it can be shown that the anti-enzymotic activity of the serum is caused by non-specific substances. This subject will by discussed under "Antileucoprotease" and it will be evident that the more recent investigators find that the anti-enzyme of the serum is not a specific antibody.

Wiens and E. Müller (103) found that the blood serum of the turtle failed to inhibit the human leucocytic enzyme but inhibited trypsin as effectively as human serum. Jochmann and Kantorowicz (55) found that turtle serum inhibited very slightly the purified leucocytic enzyme whereas it actively inhibited trypsin in from 5 to 10 per cent solution, but human serum also inhibited trypsin more completely than leucocytic enzyme.

By the action of purified enzyme from leucocytes upon Witte's peptone in the presence of sodium carbonate (less than 1.5 per cent) at 37°C. under chloroform, Jochmann and and Lockemann (57) obtained crystals of trypsin after 5 or 6 days; parallel tests with pancreatin showed the presence of characteristic crystals after one day. Leucin, tryptophan and ammonia were demonstrable according to these observers as products of leucocytic digestion of peptone and they reach the conclusion that there is a very close similarity between the enzyme of leucocytes and trypsin.

A comparison has been made by Jobling and Strouse (49) between the products of digestion obtained from casein, purified egg albumin and Witte's peptone by the action of a solution prepared from dried powdered leucocytes on the one hand and of a solution made from the powdered trypsin of commerce on the other. The quantity of proteoses with leucoprotease exceeds that with trypsin whereas products of digestion below proteoses, namely, peptone and amino acids, were much less with leucoprotease than with trypsin. No tryptophan was formed by the action of leucoprotease. The digestion with leucoprotease in their experiments did not progress as far as tryptic digestion. They found in fresh pus cells an erepsin-like enzyme which was capable of splitting petpone.

Leucoprotease and immunity. Metchnikoff (68) proposed the name microcytase for complement or alexin which is concerned in bacteriolysis because he believed that it was derived from the polynuclear leucocytes and had the characters of a proteolytic enzyme. For complement which combines with antibody or amboceptor to cause hemolysis he suggested the name macrocytose believing that it was derived from macrophages. The effect of heat upon complement indicates that it is not identical with leucoprotease on the one hand nor with protease of macrophages on the other, since these substances resist temperatures which destroy complement.

Proteolytic enzyme of leucocytes was found to have no bactericidal action by Jochmann (53) when tested with B. typhosus, staphylococci and streptococci, nevertheless, according to Jochmann, it digested typhoid and colon bacilli just as quickly as it digested fibrin. Living bacteria resisted digestion and even multiplied actively in the solution of enzyme. The enzyme of leucocytes failed to cause hemolysis of red blood corpuscles. Jochmann found that the enzyme of leucocytes, unlike trypsin, failed to destroy diphtheria toxin.

The effect of leucoprotease upon pneumococci has been studied by Jobling and Strouse (50) and for control compared with bacterial autolysis in salt solution. After incubation during twenty-four hours the turbidity of the fluid containing leucoprotease is much diminished and within forty-eight hours the fluid is almost clear though the autolyzing suspension remains cloudy. In the presence of the enzyme pneumococci are reduced to shadows after twenty-four hours and are Gram-negative whereas the autolysing microörganisms are normal in appearance and still Gram-positive. Simple chemical reactions such as total acidity and formol titration indicate that bacteria are split into cleavage products which are lower than those obtained by autolysis. Formol titration as an index of the formation of amino-acids furnished evidence that leucoprotease causes proteolysis of pneumococci.

It is noteworthy that Jobling and Peterson (39), employing Folin's microchemical method for the determination of nitrogen have found no evidence of proteolysis when immune serum and complement cause bacteriolysis of typhoid and colon bacilli although proteolysis was demonstrable when trypsin acted upon the same microörganisms; they have not described parallel experiments with leucoprotease. The clearing of a bacterial suspension under the influence of immune serum does not prove that proteolysis has occurred, for Jobling and Strouse (52) found no parallel between the clearing of a suspension and the accumulation of products of protein disintegration.

Some of the effects of leucoprotease upon typhoid bacilli have been studied by Jobling and Bull (32). Typhoid bacilli like B. coli injected into the circulating blood of dogs causes elevation of temperature, bloody diarrhea, coma and death, and the abdominal organs, particularly the intestine, are intensely congested. This toxic substance is not destroyed by heat but is precipitated with the coagulable proteins of the bacteria so that the filtrate after heating to 100°C. is not toxic. If an emulsion of bacilli is subjected to the action of leucoprotease during from 2 to 5 days, the toxic element is no longer precipitated by coagulation but is found in the filtrate obtained after heating to 100°C. The toxic substance is not specific and similar observations were made with B. coli, meningococcus and Staphylococcus aureus.

Observations of Kantorowicz (59) indicate that the resistance of living bacteria to proteolytic enzymes is referable, not to vital characters, but to anti-enzyme which is present in the bodies of the bacteria. Gram-negative bacteria lose this inhibiting property when heated to 70°C. but Gram-positive bacteria inhibit digestion even after boiling. Dried organisms retain their anti-enzymotic activity but lose their resistance when extracted with acetone. Bacteria were used in the foregoing experiments of Kantorowicz as substrate for proteolytic enzymes and digestion was measured by clearing of the suspension. Jobling and Peterson (39) measured digestion by Folin's method of nitrogen determination. They found that dried organisms resisted digestion in a degree proportional to their content of unsaturated lipoids (see anti-enzyme for leucoprotease). Substances which extract lipoids reduce the resistance of bacteria to proteolysis and the saponified extracted lipoids inhibit the digestion of casein by trypsin in a degree proportional to their unsaturation.

Bacteria, namely typhoid and colon bacilli, were found by Jobling and Peterson (39) to be so altered by immune serum and complement that they are more readily digested by trypsin. Complement alone or an excess of immune serum and complement rendered bacteria more rather than less resistant to proteolysis. Rosenow (91) noted that the intracellular digestion of pneumococci ingested by leucocytes *in vitro* varied considerably when the blood of different individuals was employed and came to the conclusion that the variation was referable to the action of the serum on the leucocytes stimulating them to increased activity. Douglas (15) treated B. pestis which undergoes phagocytosis in the absence of serum with unheated and with heated serum and subjected them to phagocytosis. Bacilli which had been treated with fresh serum underwent active intracellular digestion whereas those treated with heated serum remained intact within the substance of the leucocyte. Douglas further found that bacteria (bacillus of Friedländer) or red blood corpuscles exposed to the action of fresh serum and then washed by centrifugalization were digested by trypsin or by leucoprotease whereas bacteria or red corpuscles treated with serum heated to 60°C. remained unchanged.

INHIBITION OF LEUCOPROTEASE BY BLOOD SERUM; ANTILEUCOPROTEASE. To describe the enzymotic activities of phagocytic cells it is necessary to discuss the inhibitory factors which are concerned. The anti-enzyme of the blood serum will be discussed in relation to leucoprotease and observations concerning inhibition of trypsin will be cited in so far as they help to explain the inhibition of leucocytic enzymes. The terms anti-enzyme, antileucoprotease, etc., are used as convenient descriptive words and do not imply that the substances which inhibit are specific antibodies. The antitryptic action of the blood serum was first observed by Hildebrandt (30).

The power of the blood serum to inhibit the proteolytic activity of leucoprotease was described by Opie (79). The anti-enzyme for leucoprotease passes from the blood into an inflammatory exudate and has an important part in the phenomena of inflammation (see p. 572). The addition of blood serum or serum of an inflammatory exudate to a mixture of leucoprotease and substrate, such as denaturalized serum, gelatin or fibrin, prevents or, in smaller quantity, retards proteolysis. A temperature of 75°C. maintained during one-half hour sufficed to destroy the anti-enzymotic activity of serum, which was not impaired by a temperature of 65°C. The anti-enzyme was more effective in an alkaline (0.2 per cent sodium carbonate) than in a neutral medium but its inhibitory action was lost in the presence of acid (0.2 per cent acetic acid).

Employing the serum plate method E. Müller and Jochmann (71) found that serum inhibited the enzyme of leucocytes. E. Müller and Kolaczek (75) found no anti-enzyme in the cerebrospinal fluid; none was found in the bile nor in milk. In normal urine they found no anti-enyzme but with abundant excretion of albumin especially with chronic passive congestion of the kidneys and with chronic parenchymatous nephritis the urine inhibited the enzyme of leucocytes.

The anti-enzyme of the blood is not specific for leucoprotease obtained from the same species. Opie and Barker (84) found that leucoprotease of the dog is inhibited by serum of man, ox, dog, cat, goat and rabbit, the last mentioned being stronger than the others. Leucoprotease of rabbit was inhibited by the serum of dog and of rabbit, that of the rabbit being again the stronger. The serum of birds (pigeon, hen) on the contrary failed to exhibit anti-enzymotic action when tested with the leucoprotease of dogs. Wiens and E. Müller (103) found that the serum of the turtle failed to inhibit the proteolytic enzyme of leucocytes but inhibits trypsin almost as effectively as human serum.

Antileucoprotease was precipitated by Opie and Barker (84) with the albumin fraction of the blood serum, obtained by complete saturation with ammonium sulphate but was absent in the euglobulin fraction obtained by one-third saturation or in the globulin fraction obtained by half saturation.

By adding increasing quantities of leucoprotease to the same quantity of serum Opie (82) found that a given quantity of serum can inhibit the action of only a limited quantity of enzyme; for example, 2.5 cc. of serum completely inhibited the proteolytic activity of 20 mgm. of dried leucocytes but failed to prevent proteolysis when a larger quantity of enzyme was employed.

Anti-enzyme in the blood serum with disease. Numerous studies of the anti-enzymotic activity of the blood have been made; from the standpoint of diagnosis they have disappointed early expectations. No exhaustive review of the extensive literature of this subject will be made. Observations of interest in relation to the enzymes of leucocytes will be cited briefly.

The anti-enzymotic activity of the blood was studied by Wiens (99), (100), (101) in a considerable number of diseases; he has used the serum plate method and has performed his tests with fresh pus. With general and local pyogenic infections he had found a diminution of anti-enzyme which he attributes to an increased destruction of leucocytes in the body. Wiens and Schlecht (104) have compared the anti-enzymotic strength of the serum with the number of leucocytes in the blood and with acute infectious diseases have usually found diminution of anti-enzyme during and immediately after an increase in the number of polynuclear leucocytes; later an increase of anti-enzyme may occur. Nevertheless variations in the anti-enzyme particularly in chronic disease may bear no relation to leucocytosis.

The anti-enzyme of the blood is not infrequently increased with conditions which are unaccompanied by leucocytosis. Brieger and Trebing (10), (11) found antitrypsin increased with carcinoma and sarcoma in 90 per cent of the cases examined, and later, observing the same change with some other diseases, for example, tuberculosis accompanied by wasting and cachexia designated this increase "the cachexia reaction." Brenner (9) found that the anti-enzymotic activity of the serum was usually increased with severe anemia and chlorosis but no definite relation to the number of red blood corpuscles nor to the hemoglobin content of the blood was evident. Increase of antitrypsin was found by K. Myer (69) in association with exophthalmic goiter. Nevertheless, increase of antitrypsin is often unassociated with wasting and anemia. Gräfenberg (24) and later Thaler (98) and others found increase of anti-enzyme during pregnancy.

Sir A. E. Wright (106) has found coincident loss of antitrypsin and diminution of alkalinity in the edematous fluid at the site of infection with the gas bacillus of Welch both in man and in animals. In the blood diminution of alkalinity and increase of antitrypsin was found.

Following the administration of potassium iodide, Jobling and Petersen (41) found diminution of the antitryptic content of the blood serum, but in two instances of iodide poisoning antitrypsin was increased.

The nature of the anti-enzyme in blood serum. Increase of anti-enzymotic activity of the blood was found by Jochmann and Kantorowicz (56) when they repeatedly injected subcutaneously into rabbits an extract of dried leucocytes from human pus. Before injection one drop of serum inhibited the proteolysis of one drop of pus but after these injections serum diluted from thirty-two to sixty-four times had the same effect. There was a parallel increase of anti-enzyme both for trypsin and for leucocytic enzyme. If trypsin was mixed with a quantity of serum which just served to inhibit its activity the mixture exerted no inhibitory action upon the enzyme of leucocytes. Upon the evidence of these experiments the authors reach the conclusion that the anti-enzyme for trypsin and that for the enzyme of leucocytes are identical.

Achalme (2) injected trypsin into the peritoneal cavity of guinea pigs and found the normal inhibitory action of the serum increased. After two months Bergmann and Bamberg (7) found the anti-enzymotic activity of the serum of dogs doubled by repeated subcutaneous injection of trypsin. The anti-enzymotic activity of the serum of two dogs which received 20 cc. of 4 per cent trypsin solution was increased after 24 hours in one instance to ten times its former strength. Some observers have assumed that the trypsin acts as an antigen and brings about the formation of a true antibody. Some have maintained that the anti-enzyme of normal serum is formed in response to the presence of enzyme within the body; Wiens (99) suggests that the enzyme of the leucocytes furnishes the stimulus to its formation. Colliner (12) thinks that trypsin absorbed from the pancreas, and K. Meyer (70) that autolytic and proteolytic enzymes derived from the tissues may act as antigen.

Specificity is one of the characters of antibodies formed when an antigen has entered the body. Eisner (17) found that sera which varied in their anti-enzymotic action upon trypsin did not show corresponding variations when tested with labferment, pepsin, emulsin and lipase. Weil (105) cites evidence to show that anti-enzyme of the serum is not a specific antibody; human sera, he states, inhibit the vegetable protease papain in a constant ratio to their inhibition of trypsin. An observation of Pozerski (90) cited by Weil, is noteworthy in this connection; the serum of an animal immunized against papain contained a specific precipitin and an antibody which fixed complement but it possessed no increased ability to inhibit proteolysis caused by papain.

Increase of serum anti-enzyme following injection of trypsin is of little value as evidence of an immunity reaction, for similar increase is produced by a variety of apparently unrelated means. In animals which had received choloroform or phosphorus in quantity sufficient to produce profound intoxication Opie, Barker and Dochez (86) found an increase of proteolytic enzyme in the blood serum and in some instances complete disappearance of anti-enzyme. In animals which repeatedly received these substances in doses insufficient to cause death, there was a progressive increase of anti-enzyme. Braunstein and Kepnow (70) increased the antitrypsin of the blood by injecting an emulsion of tissue cells into the peritoneal cavity of rabbits. K. Meyer (70) found the antitrypsin of the blood increased in rabbits and dogs and also in patients after administration of thyroid tissue.

In view of the lack of evidence that the anti-enzyme of the serum is a true antibody attempts have been made to find in the serum some substance capable of inhibiting the action of proteolytic enzymes.

The antitryptic action of serum was attributed to lipoid substances by Schwarz (94). He found that an emulsion of lipoids prepared from the organs of the horse by extraction with alcohol and ether and precipitation by acetone inhibited trypsin. By extraction with ether serum was deprived of its inhibiting action but addition of lipoids restored it. He suggests that serum antitrypsin is a combination of lipoid with protein. Delezenne and Pozerski (14) had made the observation that serum treated with chloroform underwent autolysis. Sugimoto (96) found that the antitryptic action of egg white was removed by extraction with ether, petroleum ether, benzol and benzin, the effect of the last named being least. Addition of the lipoid which had been removed, of lecithin, of lipoid from brain and of lipoid from liver to the egg white extracted with petroleum ether did not restore its inhibiting action and the lipoids themselves had little effect upon the tryptic digestion of casein.

Leucoprotease and trypsin were inhibited by Jobling and Petersen (34) with sodium soaps prepared from olive oil, croton oil, cod liver oil and linseed oil. Complete inhibition of tryptic digestion of casein was caused by 0.005 gram of linseed oil soap and partial inhibition was evident with much smaller quantities. The anti-enzymotic activity of the soap was inversely related to the saturation of its fatty acid and in proportion to its iodine value. Soaps of the saturated fatty acids such as sodium stearate or palmitate did not inhibit the action of the enzymes. Saturation of an unsaturated fatty acid with iodine removed the inhibiting activity of the soap.

Studies of the nature of the serum antitrypsin have been made by Jobling and Petersen (37). The antitryptic action of serum, lost by standing under ether during 4 days was partially restored when, after evaporation of the ether, sodium hydrate was added in sufficient quantity to give the ether treated serum a slightly alkaline reaction. The anti-enzyme is removed when the serum is acidified with hydrochloric acid (e. g., 5 cc. N/10 acid added to 20 cc. of serum from the dog) and filtered through kaolin; extraction of the kaolin with sodium alcoholate furnished a solution which inhibited the action of trypsin and had about one-tenth the strength of the untreated serum. Potassium iodide was found to diminish the antitryptic action of serum. When linseed oil soap was mixed with serum its inhibiting action, like that of serum alone, was completely removed by heating during thirty minutes at 70°C. From the evidence of these experiments the authors reach the conclusion that the enzyme-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids.

Slovzov and Xenophontava (95) found that the antitryptic substance of the serum can be extracted with chloroform but not with toluol. Since fatty acids have the same antitryptic action which is reduced by iodizing they reach the conclusion that the antitryptic action of the serum is caused by lipoids.

The lipoidal nature of antitrypsin is not accepted by Cobliner (12), Meyer (70) nor Teale and Bach (97), all of whom have found that extraction of dried sera with lipoid solvents such as chloroform, ether and petroleum ether does not remove its antitryptic power. Teale and Bach cite experiments to show, contrary to the opinion of Jobling and Petersen, that lipoids are more readily extracted from the dry than from wet serum and maintain that the lipoidal solvents destroy the anti-enzymotic activity of the serum only when they bring about coagulation of protein. Their observations concerning the inhibiting properties of soaps are at variance with those of Jobling and Petersen and they reach the conclusion that the serum antitrypsin is protein in nature.

PROTEOLYTIC ENZYME OF MONONUCLEAR PHAGOCYTES (MACROPHAGES). All mammals and many lower vertebrate species possess two types of cells capable of engulfing and digesting solid particles. One type is the polynuclear leucocyte (polymorphonuclear) with fine granulations, the neutrophile leucocyte of human blood which with inflammation leaves the blood vessels and forms the chief cellular element of acute inflammatory exudates. Its enzyme, leucoprotease, has been described. The second type of phagocytic cell is larger than the polynuclear leucocyte, it has a large nucleus which is round, oval or somewhat irregular in outline but never lobulated like the nucleus of the polynuclear leucocyte, and its cytoplasm contains no granules exhibiting a specific reaction to dyes. These cells have been designated macrophages by Metchnikoff and are the endothelial leucocytes of Mallory. During the later stages of acute inflammation when the inflammatory irritant has been overcome, the number of mononuclear phagocytes increases and these cells are actively engaged engulfing and digesting within their substance polynuclear leucocytes, red blood corpuscles and other cellular elements. When recovery occurs they have an important part in removing cellular elements from the site of inflammation. Moreover, they ingest certain parasitic microörganisms such as the malarial parasites and other protozoa, the tubercle bacillus, B. leprae and some other bacteria. Their phagocytic activity in typhoid fever is well known.

The proteolytic enzyme found associated with these phagocytic cells by Opie (81), unlike the enzyme of the polynuclear leucocytes, digests with greatest activity in the presence of an acid medium. In the inflammatory exudate obtained by injecting aleuronat into the pleural cavity of a dog, the number of polynuclear leucocytes decreases with the progress of the inflammatory reaction whereas the number of mononuclear cells exhibits a corresponding increase. A comparison between the character of the exuded cells during the first five days of the inflammatory reaction and the proteolysis which they cause when completely freed from serum has shown that there is coincident with decrease of polynuclear leucocytes, decreasing capacity to digest in the presence of an alkaline reaction and coincident with increase of mononuclear cells increasing power to digest in the presence of acid. The lymph nodes adjacent to the site of inflammation, for example, with sterile pleurisy caused by aleuronat, the substernal lymph nodes, afforded an opportunity to study the enzyme of the large mononuclear cells almost wholly free from leucoprotease. In these nodes large mononuclear phagocytes occur in increasing number during the first five days of the inflammatory reaction and during this period there is increasing ability to cause proteolysis in the presence of acid but no digestion in an alkaline medium. The lymph nodes which drain the inflamed tissue and are crowded with mononuclear phagocytes exhibit much greater ability to cause proteolysis than distant lymph nodes, such as those of the mesentery, which contain few phagocytic cells. The enzyme of the mononuclear phagocytes which accumulate as the result of an inflammatory reaction has been designated lymphoprotease by Opie. It causes active digestion of protein in the presence of weakly acid reaction (0.2 per cent acetic acid) but is almost entirely inactive in the presence of a neutral or alkaline reaction. The enzyme is more susceptible to heat than leucoprotease being much impaired in activity by temperatures between 60° and 70°C. It is destroyed by higher temperatures. It is destroyed by drying after treatment with alcohol and ether. It resembles very closely the autolytic enzyme of parenchymatous tissues.

Two proteolytic enzymes have been found in experimental inflammatory exudates in the dog by Jobling and Strouse (49). One acts in an alkaline medium and is isolated by drying with alcohol and ether. The other acts in an acid medium and, unlike the alkaline-acting enzyme, is destroyed by heating to 70°C. during one-half hour. In hyperplastic lymph node in which the sinuses contained many large mononuclear cells, Longcope and Donhauser (61) found an enzyme which digested protein in the presence of weak acid.

The studies of Lord (62) and Nye (78) upon the hydrogen ion concentration favorable to the enzymes of a pneumonic exudate will be cited.

Erepsin in leucocytes. The occurrence within leucocytes of an enzyme with the character of the erepsin of Cohnheim is indicated by observations of Jobling and Strouse (49). Leucoprotease obtained after drying leucocytes in alcohol and ether failed to decompose proteoses and peptone to form amino-acids and no tryptophan was demonstrable. Fresh leucocytes split proteoses and peptone into these products in both acid and alkaline media but heat at 70°C. during one-half hour destroys this property. The leucocytes apparently contain an erepsin-like enzyme which acts upon products of the digestion caused by proteolytic enzymes. Parker and Franke (87) found that extracts of leucocytes of rabbit failed to cause any significant disintegration of protein but digested peptone to form amino-acid which was measured by van Slyke's method. Similar digestion of a suspension of typhoid bacilli was attributed to the disintegration of "bacto-peptone." They believe that these leucocytes of rabbits contain erepsin but no protease.

Petersen and Short (89) found an increase of peptone-splitting enzyme in the blood serum immediately preceding and accompanying the crisis of lobar pneumonia.

SERUM PROTEASE. Enzymes similar in their action to those of the leucocytes may be found in the blood serum and after the coagulation of the blood enzymes are found attached to the fibrin which is formed.

When blood serum was treated with chloroform by Delezenne and Pozerski (90) the serum caused proteolysis of gelatin and casein. In the blood serum of the ox Hedin (27) found a weak proteolytic enzyme which acts in the presence of an alkaline medium and is present mainly in the globulin fraction of the serum. By treatment of blood serum with weak acid (0.2 per cent acetic acid), Opie and Barker (85) demonstrated the presence of a proteolytic enzyme; the globulin fraction of the serum obtained by half saturation with ammonium sulphate contained an enzyme which digested protein in neutral or alkaline media but failed to act in the presence of acid. This alkaline-acting enzyme resembles leucoprotease which does not digest in the presence of acid. It is probable that the serum contains two proteolytic enzymes.

With chloroform poisoning in dogs of such intensity that the liver undergoes necrosis and the coagulability of the blood is diminished, Opie, Barker and Dochez (86) found that the blood serum acquires increased ability to digest protein; this increased proteolysis is referable to an enzyme which digests with maximum activity in a weakly acid medium. The enzyme which digests in the presence of an alkaline medium is not increased.

Autolysis of serum after addition of chloroform has been used by Jobling and his co-workers in a series of studies on enzyme action (42) as a measure of serum protease. This method does not serve to identify the protease with either of those which have been recognized in the serum. It is noteworthy that antitrypsin which they have simultaneously measured restrains enzymes like trypsin and leucoprotease which act in an alkaline medium but is inactive in the presence of the acid reaction which is favorable to the acid-acting protease of the serum. Protease was present in serum (33) in greatest strength in guinea pigs and rabbits and was found in cat, ox and dog. In the normal dog it was found occasionally but was constantly found with distemper, pneumonia and inanition. In normal human serum they found little or no protease. Serum protease cannot be identified with complement for it resists chloroform which destroys complement and is not destroyed by a temperature of 56°C.

Jobling and Petersen (38) found that the serum of guinea pig, rabbit and horse loses its antitryptic action when treated with kaolin, starch, agar or bacteria. They refer the toxic symptoms produced by sera so treated (anaphylatoxin) to toxic split products formed by the action of serum protease upon the proteins of the serum itself.

Several observers claim to have demonstrated in sensitized animals the presence of enzymes specific for the protein used as antigen. Jobling, Petersen and Eggstein (44) found the serum protease practically unaltered after the first injection of foreign protein; with acute anaphylactic shock, on the contrary, there was an "instantaneous mobilization" of non-specific protease and decrease in anti-enzyme. Intoxication they think is the result of cleavage products (peptones) liberated by enzyme action immediately after the introduction of the antigen. They do not regard the almost instantaneous occurrence of shock as a valid argument against this view.

The Abderhalden reaction demonstrated the presence in the serum of a proteolytic enzyme which Abderhalden has maintained has a specific action on placental tissue and is present in the serum only during pregnancy. This opinion has been much disputed and several of those who have investigated the subject have reached the conclusion that the serum itself is the source of the dialyzable products which are obtained, the placental tissue being undigested. Jobling, Eggstein and Petersen (33) maintain that protease of the serum becomes active because under the conditions of the method, the restraining influence of the anti-enzyme is removed. A full discussion of this subject is not desirable in this review.

In a series of papers Jobling, Petersen and Eggstein (43), (46), (47), (48), have described changes in serum enzymes and anti-enzymes following the injection of trypsin, kaolin, proteoses, peptones and bacteria into dogs. There was an increase of protease and usually an increase of antitrypsin which they regard as lipoid in nature. The amount of demonstrable enzyme bore no relation to variations in temperature or in count of leucocytes. They believe that a disturbance of ferment-antiferment balance in the serum may result in proteolysis of serum protein with liberation of toxic split products but they admit that these are not the sole agents of bacterial intoxication.

In the absence of profound fatal intoxication such as that caused by chloroform or phosphorus, anti-enzyme is present in the serum in sufficient quantity to restrain the action of serum protease. A complete absence of anti-enzyme, Jobling, Petersen and Eggstein state, is not essential for protease action provided some absorbing surface is present on which the relative balance of ferment-antiferment may be altered; "it seems probable that the protease action can take place in what might be termed local areas of antiferment deficiency, such as must occur at the point of contact of the serum and absorbing surface." Petersen (88) has discussed the speculative basis for recent attempts to find therapeutic use for agents which modify the enzyme and anti-enzyme of the serum.

No evidence that neutral fats, fatty acids or lipoid bodies have a part in restraining the activity of serum protease has been found by Yamakawa (107). He measured proteolysis of serum by determining with the ninhydrin reaction the amount of dialyzable products formed from serum kept in a dialyzing thimble during 16 hours at 37°C. Serum of guinea pig undergoes proteolysis when treated with chloroform, methyl, ethyl and isobutyl alcohol and acetone but after removal of the chemical activator by vacuum, dialysis or extraction with indifferent chemicals (ether or petroleum ether) the original nonautolytic state does not return. Ethyl ether, petroleum ether, benzine and toluene neither activated nor paralyzed the serum protease. Addition of cholesterol, lecithin and neutral fats such as triolein and tripalmitin failed to influence autolysis.

FIBRIN-PROTEASE. Fibrin undergoes solution when allowed to stand in weakly acid, neutral or alkaline solution. The influence of varying temperatures upon solution and the formation of products of protein disintegration have shown that enzymes are concerned in the process. Studies of Rulot (92) furnish evidence that digestion of fibrin is caused by leucocytes imprisoned in the meshes of the fibrin. Proteolysis was measured by Kjeldahl determination of the nitrogen contained in decomposition products incoagulable by heat. To obtain fibrin free from leucocytes two methods were used. Clotting of drawn blood was prevented by addition of sodium chloride and the blood was centrifuged. Subsequent dilution caused the deposition of fibrin. In the second method the blood was made incoagulable by intravenous injection of propeptone; after centrifugalization, fibrin formation was brought about by a current of carbon dioxide. Pure fibrin obtained by these methods, Rulot found, was almost insoluble in physiological salt solution but underwent proteolysis when the leucocytes obtained by centrifugalization were added to it. In fibrin from the blood Barker (5) found a proteolytic enzyme which caused autolysis of fibrin and digested a protein substrate in presence of acid neutral and alkaline media.

In the fibrin of inflammatory exudates Barker (6) showed the presence of two enzymes. Leucoprotease was abundant in exuded fibrin in the early stage of acute inflammation produced by injection of turpentine into the pleural cavity of a dog and could be separated from the second enzyme which acts in acid by drying fibrin after treatment with alcohol and ether. After the second day of inflammation leucoprotease had disappeared but a proteolytic enzyme acting in an acid or neutral medium was abundant.

INFLAMMATION. At the site of an inflammatory reaction anti-enzyme of the blood passes with the exudate into the inflamed tissue or cavity and the leucocytes are surrounded by a fluid capable of inhibiting the proteolytic enzyme which they contain. The polynuclear leucocytes engulf solid particles and digest them within vacuoles in their substance. The anti-enzyme of the surrounding fluid serves to limit the activity of the enzyme to the site in which it is effective. Should the leucocyte be destroyed the enzyme which is set free can no longer cause proteolysis. With relatively mild inflammation such as that which occurs with lobar pneumonia or with serofibrinous pleurisy there is no solution of tissue and the part is ultimately restored to normal.

Study of enzymes has served to explain many of the phenomena of resolution. At the height of the inflammatory reaction the solid elements of the exudate are polynuclear leucocytes, red blood corpuscles, in small number, other cellular elements and fibrin. Leucoprotease appears to be chiefly concerned with the digestion of protein particles, such as bacteria engulfed by the polynuclear leucocytes. The mononuclear phagocytes which accumulate during the later stage of an inflammatory reaction which is proceeding toward recovery digest within their substance polynuclear leucocytes, red blood corpuscles and other cells. This phenomenon occurs not only at the site of inflammation but in the sinuses of the regional lymphatic nodes to which cellular elements as well as fluid are carried by way of the lymphatics. The enzyme of the large mononuclear phagocyte resembles the autolytic enzymes widely distributed in the parenchymatous tissues and is doubtless capable of causing autolysis and final disappearance of the macrophage under suitable conditions. It is often assumed that autolysis of the exudate is caused by the leucoprotease of polynuclear leucocytes but the correctness of this view has not been established.

When experimental pleurisy is produced by intrapleural injection of a sterile inflammatory irritant such as turpentine, serum accumulates and fibrin is deposited on the pleural surfaces. Accumulation of fluid reaches a maximum after 3 or 4 days and subsequently fluid rapidly subsides so that it has disappeared after 6 or 7 days. Reactions showing the presense of peptone and albuminoses were obtained by Opie (83) on and after the third day. Leucoprotease was present in the fibrin which was removed from the cavity during the first few days of inflammation and perhaps at this period had a part in causing its solution but later at a time when fluid had disappeared from the cavity this enzyme was no longer demonstrable and the fibrin which in diminishing amount was present in the pleural cavity during the next five or more days underwent autolysis only in the presence of weak acid. Final disappearance of fibrin is evidently brought about by an enzyme which has the characters of lympho-protease and resembles the autolytic enzymes of the tissues. It is difficult to determine what are the factors which bring this enzyme into action. It is noteworthy that the alkalinity of the exudate was less than that of the blood and diminished slightly with the progress of the inflammatory reaction.

When an inflammatory reaction is of such intensity that leucocytes accumulate in immense numbers and solution of tissue and of fibrin occurs, suppuration is established and resolution with restoration to normal is no longer possible. Opie (82) found that disintegration of polynuclear leucocytes in a purulent exudate sets free leucoprotease in quantity sufficient to completely overcome the anti-enzyme of the exuded serum. Both the whole pus and its fluid part separated by centrifugalization from the pus cells was now capable of causing proteolysis *in vitro*. The proteolytic activity of the exudate unrestrained by anti-enzyme explains the solvent action of pus for injured tissue and fibrin. An inflammatory irritant such as turpentine injected into the pleural cavity of the dog, where fluid rich in anti-enzyme readily accumulates, causes a serofibrinous inflammation which undergoes resolution with restoration of the cavity to normal. An equal quantity of the same irritant injected into the subcutaneous tissue where fluid accumulates with difficulty causes on the contrary very extensive suppuration with solution of tissue and healing by scar-formation.

In pus produced by pyogenic cocci, for example, with acute peritonitis, E. Müller and Kolaczek (75) found that the anti-enzyme was "saturated" by enzyme derived from leucocytes which had undergone disintegration; the fluid overlying the cells after centrifugalization exhibited proteolytic activity.

Since the normal spinal fluid, unlike other body fluids, contains neither enzyme nor anti-enzyme, Dochez (13) has studied the changes which occur with inflammation. With epidemic meningitis anti-enzyme may enter the spinal fluid but quickly leaves it. With the more severe inflammation caused by pneumococcus or streptococcus leucoprotease derived from disintegrating polynuclear leucocytes gives the fluid proteolytic activity which may itself act as an irritant and increase the severity of the disease.

In the exudates from acute arthritis, pleurisy, and wounds of war, E. Zunz (109) at La Panne in Belgium studied the relation of the antiproteolytic property of inflammatory exudates to their alkaline reserve. The fluid was separated from the cells of the exudate and the quantity of this fluid necessary to inhibit a standard solution of trypsin was determined and designated the antitryptic index. The hydrogen ion concentration of the serum entirely freed from carbon dioxide was determined by the method of Marriott. The tryptic index and the alkaline reserve of serous or hemorrhagic exudates never exceeded and were almost always less than those of the blood serum of the same individual. The fluid from purulent exudates did not usually inhibit tryptic digestion and on the contrary digested coagulated egg white but occasionally this fluid had a slight antitryptic action. The alkaline reserve of seropurulent and purulent exudates was almost always less than that of serous exudates; increase of alkaline reserve was often parallel with an increase in the number of leucocytes and a diminution of the antitryptic index but the alkaline reserve tended to return to normal when the leucocytes diminished and the antitryptic index increased.

In serous exudates Zunz has found that diminution of complement is often parallel with diminution of antitryptic activity but this relation is not constant. In purulent exudates with complete or almost complete loss of antitryptic action complement has disappeared. Since the enzyme of leucocytes is present in excess the observation furnishes evidence, were more needed, to show that complement (microcytase) and proteolytic enzyme of leucocytes are not identical. Opie found that opsonin disappeared from an inflammatory exudate when it became purulent. It is probable that the loss of these bodies is referable to the action of unrestrained enzyme.

PNEUMONIA. The autolysis of the consolidated lung of lobar pneumonia was studied by Friedrich Müller (77); albumoses, leucin, tyrosin and other products of protein disintegration were formed at body temperature and nuclei disappeared as the result of decomposition of neucleins. Flexner (22) found that autolysis took place much more quickly and perfectly in lungs in the stage of gray than of red hepatization and attributed the difference to the greater number of leucocytes with gray hepatization. Autolysis of the lung in instances of unresolved pneumonia was slow and incomplete.

By means of the serum plate method Lord and Nye (64) have studied the relation of enzyme to anti-enzyme at different periods of the disease. Sputum and exudate obtained at autopsy in the later stages of lobar pneumonia erode the serum plate. In the early stage of the disease proteolysis does not occur but if the cellular material obtained from the exudate is separated from the serum which accompanies it active digestion occurs. In this early period enzymes are inhibited by anti-enzymes. The author suggests that excessive impairment of circulation or excess of enzyme may result in disintegration of the pulmonary framework and abscess formation whereas excess of anti-enzyme may prevent resolution.

The hydrogen ion concentration favorable to the action of proteolytic enzymes contained in the cells derived from the pneumonic lung has been studied by Lord (62) and by Nye (78). They have used the serum plate, gelatin and peptone as substrate. The proteolytic enzymes are active with hydrogen ion concentrations between 7.3 and 6.7 and are inactive with higher, that is, more acid concentrations. Lord found an enzyme which splits peptone to form amino-acids; it acted with hydrogen ion concentrations between 8.0 and 4.8 but was most active between 6.3 and 5.2. He suggests that there is a gradual increase of the hydrogen ion concentration of the pneumonic exudate; the proteolytic enzyme or enzymes which act in a weakly alkaline and weakly acid medium are perhaps inhibited by the increasing hydrogen ion concentration but the peptone splitting enzyme is further activated when the hydrogen ion concentration of the exudate is increased to the range of its optimum activity.

In the blood serum of patients with lobar pneumonia Ascoli and Bezzola (3), using the casein method of Fuld and Gross, found an increase of antitryptic activity in the earliest stage of the disease; this increase is maintained for a time and is followed by a decrease which occurs after the crisis. They have suggested that these changes are referable to a kinase which is derived from leucocytes and activates pancreatic trypsin; upon the basis of this speculation increase of antitrypsin is regarded as a reaction which follows the liberation of kinase by disintegration of leucocytes in the pneumonic exudate. Using the serum plate method to test the strength of serum anti-enzyme for enzyme of pus cells Bittorf (8) found decrease of anti-enzymotic activity during the crisis followed by a considerable increase immediately after crisis and subsequently return to normal.

A study of *a*, the antitrypsin of the serum, its effect upon tryptic digestion being measured by Folin's method for nitrogen determination; of *b*, serum protease, measured by autolysis of serum after treatment with chloroform; and of *c*, non-coagulable nitrogen-containing substances of the blood, has been made by Jobling, Petersen and Eggstein (45). They find that the crisis of pneumonia is usually accompanied by *1*, a decrease of the serum antiferment which before the crisis is increased; by *2*, a mobilization of non-specific protease in the serum; and by *3*, a decrease in the non-coagulable nitrogen and proteoses in the serum. They suggest that toxic split products of fibrin and of leucocytes rather than toxic products of the pneumococcus dominate the symptomatology of lobar pneumonia; crisis is perhaps the beginning of active autolysis depending upon an altered relation between ferment and antiferment so that with rapid autolysis toxic materials are destroyed.

In pneumonia that terminates by crisis or by lysis Petersen and Short (89) found that an increase of ereptase (peptidase, erepsin) invariably precedes or accompanies the change of clinical symptoms associated with crisis or lysis, but in instances in which death occurs the ereptase in the serum is usually less than that in the serum of a normal individual.

LEUCEMIA. The presence of incoagulable albumose-like substances in leucemic blood removed from the body after death was recognized by E. Ludwig (65). Erben (20) found that peptone and albumoses were not present in the blood when it was drawn but appeared after plasma and leucocytes separated from red corpuscles had stood in the incubator under aseptic conditions during three days. When the precipitate formed by treating leucocytes and plasma with alcohol was dried and extracted with glycerine the extract digested fibrin but lost this property when boiled. Normal blood and blood from cases of lymphatic leucemia did not undergo the change found with spleno-myelogenous leucemia. In cases of myelogenous leucemia, Schumm (93) demonstrated that products of protein disintegration were not present in the blood when it was removed from the body but appeared after the blood had been allowed to stand under chloroform. He attributes the change to a proteolytic enzyme.

E. Müller and Jochmann (71) showed that a serum plate was excavated by the blood of myelogenous leucemia when kept at 55°C. The blood of lymphatic leucemia did not produce these changes. Müller and Jochmann and Eppenstein (18) showed that the enzyme was in the cells of the blood and was inhibited by the serum.

Using the serum plate method, Jochmann and K. Ziegler (58) found that the bone marrow and spleen from cases of myeloid leucemia caused very active digestion; lymph nodes caused digestion in proportion to the myeloid transformation which they had undergone. Bone marrow of normal individuals caused active digestion of the serum plate, spleen caused slight digestion and lymph node none. Organs from cases of lymphatic leucemia and pseudo-leucemia, they state, cause no more digestion than those from normal individuals.

Studies have been made to determine if the large basophile cells of acute leucemia contain a proteolytic enzyme similar to that present in the myelocytes of myelogenous leucemia. In a case designated acute myeloid leucemia, K. Ziegler and Jochmann (108) found that half of the nucleated blood cells in the bone marrow and spleen were pure basophile cells or transitions between these and granular myelocytes; these tissues caused very active proteolysis of serum plates. Eppenstein (19) found no difference in enzymotic activity between the large lymphocytes of the blood from a case of acute lymphatic leucemia and the small lymphocytes from the chronic type of the disease. Müller and Jochmann (74) state that the blood cells in a case of acute lymphatic leucemia acted upon coagulated blood serum in the same manner as polynuclear leucocytes. Longcope and Donhauser (61) studied a case classified as acute lymphatic leucemia because the blood contained large mononuclear cells of the type of large lymphocytes with abundant faintly basophilic usually nongranular cytoplasm. Nevertheless a few neutraphilic myelocytes were found in the blood. The cells from the blood drawn during life and from lymphatic nodes obtained at autopsy caused proteolysis, measured by the Kjeldahl method, in an alkaline medium, and in this respect differed from the small lymphocytes of chronic lymphatic leucemia which caused no digestion and from the large mononuclear ("endothelial") cells of a hyperplastic lymph node which digested only in the presence of acid.

With myelogenous leucemia Jochmann and Kantorowicz (56) and Wiens (101) found no definite alteration of the anti-enzyme of the blood so that with a very high leucocytic count it may be normal. Wiens (102) suggests that enzyme set free by destruction of granular leucocytes may stimulate formation of anti-enzyme so that the two are balanced. In fatal cases, he states, there may be a loss of anti-enzyme before death so that the serum may cause proteolysis.

TUBERCULOSIS. Caseous material from tuberculous lesions or the lung with tuberculous pneumonia did not undergo the active autolysis which Friedrich Müller (77) observed with lobar pneumonia. Heile (29) thought that the failure of caseous material to undergo absorption was due to absence of enzyme rather than to insolubility of caseous material. He found that the contents of a "cold abscess" did not digest fibrin but after injection of iodoform in glycerine polynuclear leucocytes appeared and the exudate now dissolved fibrin. E. Müller and Jochmann (71) found that fluid from a "cold abscess" unlike pus of acute inflammation did not excavate the surface of serum plates incubated at 55° to 60°C.

The presence in tuberculous tissue of a proteolytic enzyme digesting in a weakly acid medium was demonstrated by Opie and Barker (85). This enzyme resembled that of the large mononuclear phagocytes and digests protein more actively than the similar enzyme of parenchymatous organs such as the liver. The presence of tubercles within the liver increases the proteolytic activity of the tissue in neutral or acid media. These observations are in accord with the well-known histological characters of the tubercle which in great part consists of mononuclear cells capable of acting as phagocytes. The enzyme of tuberculous tissue is most abundant at a time when caseation is beginning but with advance of caseation its activity diminishes and with complete caseation total disappearance of enzyme seems to occur. Leucoprotease digesting protein in the presence of an alkaline reaction was found only when the tuberculous tissue was first formed and, examined microscopically, was found to contain some polynuclear leucocytes.

No autolysis of caseous material from lymph nodes first dried and ground and then suspended in water was found by Jobling and Petersen (36) unless the tuberculous nodes had become secondarily infected. When the lymph nodes were infected, autolysis occurred in the presence of alkaline and of acid reaction but was much more active in the latter instance. Affected areas in lungs with caseous pnemonia were freed as much as possible from less involved lung tissues, dried, ground, suspended in water and subjected to autolysis. The authors assume that the caseous material was not completely freed from inflammatory exudate. Autolysis was active in the presence of acid but absent when the medium was alkaline. With the purpose of removing inhibiting substances from the material under examination Jobling and Petersen first treated some of it with acidified alcohol, centrifugalized, washed with alcohol and ether and dried. It then underwent autolysis in the presence of an alkaline reaction. The authors do not discuss the origin of this enzyme which, like leucoprotease, acts in an alkaline medium, and do not appear to have examined the tuberculous lung tissue to determine if it was secondarily infected with pyogenic microörganisms or contained polynuclear leucocytes.

From the bodies of tubercle bacilli Jobling and Petersen (35) have obtained an extract which inhibits the action of trypsin and of leucoprotease. An extract of tubercle bacilli made with ether and alcohol did not inhibit and an extract was prepared as follows: the ether-alcohol extract was dissolved in ether, precipitated with acetone, evaporated to dryness and saponified with alcoholic potash; the resulting soap was dissolved in water and repeatedly extracted with petroleum ether, treated with hydrochloric acid to liberate fatty acid, taken up with ether, washed with water and resaponified. An antitryptic agent was obtained. Though the soap obtained had a lower iodine value than soaps previously prepared from linseed, olive and cod liver oils, it had a greater inhibiting activity. This inhibiting action was lost when the soap was saturated with iodine. The same observers have extracted from caseous material of tuberculous lymph nodes and of the lung with caseous pneumonia, inhibiting agents with similar characters. They believe that these observations explain the failure of caseous material to undergo disintegration through the action of phagocytes or by autolysis but they do not assume that the inhibiting agent is wholly derived from the tubercle bacillus for they suggest that autolysis of the necrotic material of an anemic infarct is prevented by similar factors.

It is noteworthy that the observations which show that soaps of unsaturated fatty acids are anti-enzymotic agents have reference to trypsin and to leucoprotease. There has been no suggestion that trypsin is present and it is well known that polynuclear leucocytes in the absence of secondary infection are an inconspicuous constituent of tuberculous tissue and are not usually found in or about a caseous focus or an anemic infarct. Jobling and Petersen (41) have assembled evidence to show that potassium iodide which in moderate doses, they find, diminishes the antitryptic activity of the blood serum, hastens the softening of caseous material. A similar explanation is offered to explain the absorption of syphilitic gumma caused by potassium iodide. Nevertheless there are no observations to show that enzymes such as leucoprotease which are inhibited by the anti-enzyme of the blood serum are concerned in the absorption of the tubercle or of the gumma.

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THE PHYSIOLOGY OF CREATINE AND CREATININE

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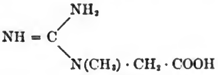
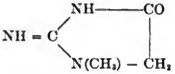
Creatine is α-methylguanidine-acetic acid, or methylglycocyamine, and may accordingly be looked upon as a derivative primarily of acetic acid, or of glycocoll, sarcosine (methylglycocoll), guanidine, methylguanidine, or glycocyamine (guanidine-acetic acid). From the standpoint of purely animal biochemistry one of its most distinguishing structural characteristics is its N-methyl radicle, which brings it into relation with the betaines and with choline. Its guanidine group, on the other hand, suggests a connection with arginine, the only known derivative of guanidine among the products of protein hydrolysis.

Creatinine, 2-imido-5-keto-3-methyl-tetrahydroimidazole, or methylglycocyamidine, is the internal anhydride of creatine, to which therefore it is related in the same way as glycocyamidine to glycocyamine. The ring resulting from the loss of a water molecule is built upon the imidazole (or glyoxaline) nucleus, so that creatinine possesses, besides the relations dependent upon its derivation from creatine, others with such biochemically important imidazole derivatives as histidine and the purines. Among the latter guanine, 2-amino-6-oxypurine, may claim a two-fold connection with creatinine, for in addition to the imidazole nucleus common to every purine its structure includes, as part of its pyrimidine complex, a guanidine residue.

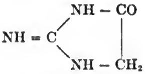
The structural interrelationships of the compounds mentioned are made obvious in the accompanying formulae:



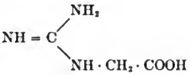
Creatine



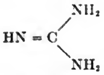
Creatinine



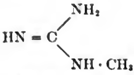
Glycocyamidine



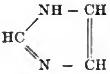
Glycocyamine



Guanidine

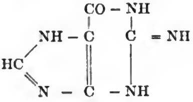


Methylguanidine



Imidazole

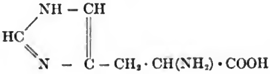
$NH_2 \cdot CH_2 \cdot COOH$
Glycocoll



Guanine

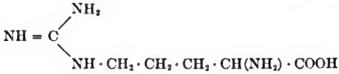
$NH(CH_3) \cdot CH_2 \cdot COOH$
Sarcosine

$HO \cdot N(CH_3)_3 \cdot CH_2 \cdot COOH$
Glycocoll-betaine



Histidine

$HO \cdot N(CH_3)_3 \cdot CH_2 \cdot CH_2OH$
Choline



Arginine

Of the chemical properties of creatinine and creatine two are of special importance from the point of view of the biochemist.

The first is the power of creatinine to react with alkaline sodium picrate with the formation of an intensely red reduction product, probably sodium picramate. Upon this reaction, first described by Jaffé (105), Folin (61) based a colorimetric method for the determination of creatinine, from the introduction of which in 1904 all sure progress in the quantitative study of creatine-creatinine metabolism can be dated. Folin (61), (64) further utilized the reaction in a method for the indirect determination of *creatine*, involving its conversion into creatinine by heating under suitable conditions (see below) with an acid. In their original form these methods were adapted only to the determination of relatively considerable quantities of "total" or preformed creatinine, but they have been variously modified, by Folin himself and by others, so as to be applicable to the analysis of very small volumes or very great dilutions of urine (184), (67), or to the determination of creatine and creatinine in muscle (160), (184), (68), (12), (145), (107), (14), (15), blood (184), (68), (148), (214), (51), (79) (71), tissues generally (184), (68) and milk (68). The original procedure for the conversion of creatine to creatinine has also received various modifications (169), (67), (27), (13), (83). While the application of these methods is by no means free from possible errors and fallacies, they have constituted, as a whole, one of the most useful and convenient weapons of biochemical research ever forged.

The second important property of creatine and creatinine is the readiness of each to undergo, under appropriate conditions, a transformation into the other. In alkaline media the transformation is mutual, reversible and fairly rapid. Thus in N/10 NaOH a 0.38 per cent creatine solution reaches equilibrium in about 2 days at 26°, and contains then about 0.1 per cent of creatinine (82). The determination of the equilibrium constant $\left(K = \frac{[\text{creatine}]}{[\text{creatinine}]}\right)$ is rendered difficult by the fact that alkalis bring about also a gradual decomposition of creatinine; but it has been estimated indirectly to have approximately the value 2.12 (82). In a pure aqueous solution at 36° equilibrium is attained only after 11 months (147), but in 21½ hours at 98° (83); the molecular ratio of creatine to creatinine is then only 0.4 (82). In acid solutions the equilibrium point is so far on the side of creatinine—a fact to be explained by the greater basicity of that substance (82)—that the reaction is practically irreversible; in other words acids convert creatine completely into creatinine, but leave the latter unaffected. The velocity of the conversion, for any given concentration of creatine, depends on the temperature and on the concentration of hydrogen ions. In normal HCl the change, for small concentrations of creatine, is complete in 15 days at 26°, and within 24 hours at 60° (82), (83); in N/2 HCl it takes about 3 hours at 98° (64), and 15 minutes at 117° (25).

BIOLOGICAL DISTRIBUTION OF CREATINE AND CREATININE. Creatine and creatinine are known with certainty only as products of the metabolism of vertebrates. Reports of the presence of creatinine in the culture media of bacteria (10), (75), (60), (178), (179), (137), or, along with creatine, in the tissues of *Abalone*, a mollusc (8), are based as yet only upon imperfectly specific color reactions.

Creatinine is a constant constituent of the urine of mammals, occupying as a rule a place among the organic nitrogenous catabolites second in quantitative importance only to that of urea. In the urine of birds it is relatively less abundant, being replaced there largely, although apparently not entirely, by creatine (156), (196). Reptiles appear in this respect to resemble birds (124). The meager data available regarding fishes (44), (45) seem to put elasmobranchs with the mammals, teleosts with the birds. The position of the amphibia is unknown; van der Heyde (89) could find neither creatinine nor creatine in the urine of hibernating frogs. Most adult mammals—ruminants forming, it would seem, an exception (153)—excrete under normal circumstances either no creatine at all or only irregular traces.

Creatinine is believed to occur also, although in minute quantities only, in the tissues and fluids of the body. Typical examples of the concentrations which have been reported will be found in table 1. The data are derived entirely from micro-analytical applications of the Folin method, and are undoubtedly subject to considerable error (102), (214), (79). In the particular case of blood Behre and Benedict (20) have indeed recently produced evidence tending to show that the error of the determination greatly exceeds any possible quantity of creatinine that might be present. Their findings should probably be regarded as still requiring confirmation; but if they should prove to be correct for blood, grave doubt will be thrown upon the supposed concentration, and even the existence, of creatinine in other tissues also. For the present the creatinine figures of table 1 will be assumed to possess at least a relative value; but it must be recognized that any conclusion of which they form an essential premise lies under some suspicion.

Creatine is a characteristic component of certain special tissues, and is possibly, as indicated again by the quantitative data of table 1, not altogether lacking in any. Its presence has been unequivocally demonstrated, either by actual isolation in substance or by production, after dehydration, of the characteristic creatinine zinc chloride, in the cases of striated muscle of the vertebrates generally (including mammals, birds, amphibians, fishes, and the lamprey), of mammalian heart muscle, of brain (21), and of the blood as a whole (20). It has been shown, by the same criterion, that blood serum contains certainly either creatine or creatinine or both (121). For the other tissues named in table 1 (including, as far as I have been able to discover, the smooth muscles of vertebrates) the evidence for the occurrence of creatine is limited to the positive outcome of the Jaffé test after treatment of the tissue or its extract with acids. Where this test indicates really notable quantities of creatine, as with the testis, it is perhaps justifiable to assume that actual creatine could be obtained from the tissue if anyone took the trouble to seek it; but where its reported concentration is as low as it is with smooth muscle and with the majority of the non-muscular tissues, a certain amount of scepticism regarding its presence is excusable.

TABLE 1

Creatine and creatinine content of tissues

(Mgm. per 100 gm.)

TISSUE	ANIMAL	CREATINE	CREATININE	AUTHORITY
Striated muscle	Man (adult)	393–430	10.0–11.5	(184)
Striated muscle	Man (1¾ years)	331		(46)
Striated muscle	Man (at birth)	190		(168)
Striated muscle	Rabbit	494–540	3.4– 9.2	(140)
Striated muscle (white)	Rabbit	404–578		(159)
Striated muscle (red)	Rabbit	277–380		(159)
Striated muscle	Rabbit (adult)	430		(133)
Striated muscle	Rabbit (39 days)	390		(133)
Striated muscle	Rabbit (19 days)	316		(133)
Striated muscle	Rabbit (9 days)	228		(133)
Striated muscle	Rabbit (7 days)	191		(133)
Striated muscle	Rabbit (fetal)	Trace		(133)
Striated muscle	Cat	421–580	2.3– 8.0	(69) (70)
Striated muscle	Pig	364–410		(21)
Striated muscle	Ox	421–530	10.3–12.7	(21) (184)
Striated muscle (fetal)	Ox	26–290		(21)
Striated muscle	Sheep	406–419		(93)
Striated muscle	Horse	376–395		(93)
Striated muscle	Dog	408–488	5.0–15.6	(69) (184)
Striated muscle	Guinea pig	370		(133)
Striated muscle	Rat	470		(140)
Striated muscle (breast)	Hen	408–481	5.0– 5.3	(35) (140)
Striated muscle (leg)	Hen	348–368	1.6– 1.9	(35) (140)
Striated muscle	Frog	364–399		(175)
Striated muscle	Turtle	236–339		(69)
Striated muscle	Skate	280		(133)
Striated muscle	Cod	350		(133)
Striated muscle	Carp	421	77	(151)
Striated muscle	Shark	655	134	(151)
Striated muscle	*Phycis*	295–313		(35)
Striated muscle	Lamprey	290		(133)
Cardiac muscle	Ox	240–255		(21)
Cardiac muscle (fetal)	Ox	31–54		(21)
Cardiac muscle	Dog	210–327		(69)
Cardiac muscle	Cat	221–333		(69)
Cardiac muscle	Rabbit	186–291		(69)
Cardiac muscle	Sheep	208–339		(69)
Cardiac muscle	Hen	166–190		(69)
Cardiac muscle	Turtle	70–109		(69)

TABLE 1—*Concluded*

TISSUE	ANIMAL	CREATINE	CREATININE	AUTHORITY
Smooth muscle				
Uterus	Cow	35–50		(21)
Uterus (at term)	Cow	98–104		(21)
Uterus	Human	52		(21)
Uterus (at term)	Human	89		(21)
Uterus (post-partum)	Human	51		(21)
Colon	Rabbit	36		(21)
Small intestine	Rabbit	27		(21)
Gizzard	Goose	70		(156)
Bladder	Ox	99–130		(35)
Brain	Dog	108		(107)
Brain	Ox	103		(107)
Testis	Bull	195		(14)
Testis	Dog	181		(107)
Testis	Sheep	209–215		(14)
Liver	Dog	21–28	2.8	(14) (184)
Pancreas	Ox	14–23		(21)
Kidney	Ox	14–20		(21)
Spleen	Dog	15–23	4.0 (Cat)	(21) (140)
Thyroid	Calf	13	1.1	(21) (140)
Thymus	Calf	11		(21)
Blood	Man	2.1–4.9	0.7–1.3	(103)
Blood	Hen	11	0.1	(69a)
Milk	Cow	2.0–2.6	1.0–1.5	(53)
Milk	Human	1.9–3.9	1.0–1.6	(54)

Table 1 includes not by any means all the data to be found in the literature upon the creatine content of tissues, but such a selection as may illustrate the chief peculiarities of its distribution. It will be seen that while noteworthy concentrations of creatine are to be found in the brain, the testes and the gravid uterus, with quantities greater still in the myocardium, no other tissue contains so high a percentage as voluntary muscle. Taking into account the average relative mass of the different organs, Bürger (32) has calculated that about 98 per cent of all the creatine in the human body is carried by the muscles, and about three fourths of the small remainder by the brain.

The creatine concentration of the muscles of a given species, among individuals chosen at random, varies within limits that are frequently rather wide; but there are cases like that of the rabbit (140), (164) where, as the consequence perhaps of uniform conditions of feeding and environment, the muscle creatine has been found to be very nearly a constant. The variability, such as it is, does not obscure the fact that there is a certain average concentration of creatine fairly characteristic for each species.

The figures reproduced furnish evidence for some other interesting generalizations. The creatine content of muscle is not the same at all ages, but rises steadily during fetal and post natal growth till it attains the maximum characteristic of the adult. There is more creatine in the quickly contracting pale muscle than in the slowly contracting red, a difference exhibited alike by mammal and by bird. The muscle creatine of warm-blooded animals is generally speaking higher than that of the more sluggish cold-blooded, although some of the data for fish supply striking exceptions. Smooth muscle has a very much lower concentration of creatine than striped; and the gravid uterus a higher one than the non-gravid. All these facts point in one direction. They indicate very strongly that creatine is a substance with a useful function, a function in some way connected with that capacity for rapid and powerful contraction which is the most important property of striated vertebrate muscle.

CREATININE AS A CATABOLITE. Whatever the rôle of creatine may be, there is no doubt that creatinine is a mere waste product. This is demonstrated by the fact that when it is administered to animals, whether parenterally (202), (143), (176), (126) or by mouth (93), (64), (94), (216), (72), (202), (176), (172), (74), the greater part, up to 80 per cent or more, is promptly excreted. The missing 20 per cent or so has in some instances been traced to the muscles, where it has doubtless undergone the reversible transformation into creatine (143). None of it is ever converted within the body into urea or ammonia (64), (70), (172) or other known product of further degradation. Not only therefore is creatinine a waste material, but it is a terminal and not merely an intermediate product of catabolism.

The daily output of this waste product, upon a diet free from either creatine or creatinine itself, is for each individual a practically constant quantity, entirely independent of the amount of protein in the food, or of the total nitrogen catabolism, or of variations in the volume of the urine. This striking and fundamental law of metabolism, the discovery of which constitutes the real starting point of modern advance in the physiology of creatinine, was first announced in 1905 by Folin (62), and has been fully confirmed by many later investigators (93), (43), (109), (181), (177). The "constancy" of the daily output is of course not absolute. It would appear from data scattered throughout the literature that the day to day variations may even amount to 20 or 25 per cent; but they do not often exceed 10 per cent, and are often for long periods together very much less. In any case they bear no relation to nitrogen output or diuresis.

According to Shaffer (182) the rate of elimination is uniform not only from day to day but even from hour to hour. The data of Klercker (109), Hoogenhuyze and Verploegh (95), (94), and Neuwirth (150) show no such regularity; and Schulz (177), who collected his own urine in two-hour periods for many consecutive days, found periodic variations with definite maxima, persistent even during fasting, at 9-11 a.m., 3-5 p.m. and 9-11 p.m. Schulz's data show also that the average of the night hours is lower than the average for the day. His observations were limited to one individual and it is not certain that they can be generalized. The lower rate of elimination during the night has been observed by Powis and Raper (162) and by Campbell and Webster (36), and is generally evident in the extensive data of Hoogenhuyze and Verploegh.

While the daily creatinine output is approximately a constant for the individual, it varies decidedly from one individual to another. Folin (62) recognized that the variations stood in some relation to the weight of the subject, and Shaffer (182) introduced the practice of expressing this relation by means of the "creatinine coefficient"—the number of milligrams of creatinine (or creatinine nitrogen) excreted daily per kilogram of body weight. This coefficient, expressed in terms of creatinine, varies in "strictly normal" human adults of the male sex from 18 to 32 (182); though possibly the lower limit of normality should be reduced to 15. In normal women the figures have the decidedly lower range of 9 to 26, averaging only 15.6 (203); it is probable, as Shaffer thought, that this is not an effect of sex in itself, for individual women whose muscles have been developed by gymnastic training have coefficients comparable with those of men (203). Infants and children have lower coefficients still; 6.7 to 10 at 10 to 14 days (9), and 9 to 17 at 5 to 13 years (118). The considerable range of the figures in every group shows that the relation between creatinine output and total body weight is not one of strict proportionality. What other factors intervene will be considered presently.

Metabolic significance of urinary creatinine. The constancy of the creatinine output and its utter lack of relation to the total protein metabolism were interpreted by Folin (63) as indicating that creatinine is the product of a special quantitatively unvarying form of protein metabolism, described as constituting an "essential part of the activity which distinguishes living cells from dead ones," named "*tissue* or *endogenous* metabolism," and sharply differentiated from the variable "exogenous" metabolism of the food protein. Apparently this special type of metabolism was conceived of as taking place in all the living protoplasm of the body. Differences in the creatinine coefficient were ascribed to differences in adiposity, that is, to differences in the relative amount of metabolically inert substance in the body; and the conclusion was implied, if not expressly drawn, that the creatinine output depends on the mass of active protoplasmic tissue. In his latest exposition of his views (70) Folin, indeed, states positively that he regards creatinine as an "index or measure of the total normal tissue metabolism."

In its main point, the idea of creatinine as the product of a special tissue metabolism and as having no relation to the catabolism of food protein, this conception has met with practically universal acceptance. In its details it has been variously modified. Shaffer (182) showed that there is a close parallelism between the creatinine coefficient of the individual and his muscular development, strength, or "efficiency." He suggested therefore that creatinine "is derived from, and an index of, not the total tissue or endogenous catabolism, but of one special process of this catabolism," taking place "largely if not wholly in the muscles," upon the intensity of which depends "the muscular efficiency of the individual." Similar views were expressed by Spriggs (186). There is of course an obvious probability that the production of creatinine should take place mainly in the tissue which is richest in creatine; but Shaffer's conclusions were reached independently of this consideration.

There are certain developments of Shaffer's view in which the direct derivation of creatinine from muscle creatine is taken as granted or proved. The first of these is the hypothesis of Pekelharing and Hoogenhuyze (159), which attempts to define more precisely than Shaffer did the relation between creatine or creatinine and muscular "tone." On this hypothesis the chemical transformations involved in the maintenance of tonus are considered to be entirely different from those associated with the rapid tetanic contractions of voluntary movement; while the latter consist preëminently in the oxidation of carbohydrate, the former are thought to include changes of nitrogenous material and in particular the formation of creatine. The output of creatinine is therefore determined by the state of tone of the muscular system.

This theory was supported by a great deal of experimental evidence tending to show that muscles in a state of "tonic" contraction, brought about in a great variety of ways, gained in creatine, and that sustained contraction or "tonus" such as is involved in the maintenance of the Prussian military posture is accompanied, in contrast with ordinary muscular effort, by an increased output of creatinine (161). It may be questioned whether all the forms of continuous contraction studied by Pekelharing and Hoogenhuyze were examples of exaggerated "tonus" in the strict sense of the term. In any case Brown and Cathcart (31) obtained comparable increases of creatine in frog muscles stimulated to contract in the ordinary way, and Schulz (177) failed to confirm the alleged special effect upon creatinine output of sustained contraction in man. It is more than doubtful therefore whether the distinction drawn between the chemical mechanism of tonus and that of voluntary contractions can be taken as established. Some relation, we have seen already, probably does exist between the functional efficiency of striped muscle (one factor in the maintenance of which is tonus) and its creatine content; but as far as the evidence goes it is just as likely that tonus is dependent on creatine, as that creatine and creatinine are products of tonus.

The theory just discussed has this in common with that of Shaffer that it makes urinary creatinine an index of muscular tone. According to Myers and Fine (140) it is simply an index of muscle creatine. In a manner this is a synthesis of the two precedent hypotheses, which somewhat to its advantage leaves open the question of the significance of the creatine. Myers and Fine observed that there is an approximate proportionality between the creatine concentration in the muscles (or entire body) of an animal and its creatinine coefficient. Thus the average creatinine coefficients of the rabbit, man and dog are respectively 38.4, 24.2 and 22.5; the average percentages of creatine in the muscles of these animals are, in the same order, 0.52, 0.39 and 0.37. The coefficients stand in the ratio 1.7 : 1.07 : 1.0; the creatine concentrations in the ratio 1.4 : 1.05 : 1.0. Comparison of different individuals within a single species leads to a similar result: for 5 rabbits, with coefficients between 36 and 37, the average concentration of creatine in the entire body was 0.170 per cent; for 5 others with coefficients of 38 to 41 (average 40.3), the average creatine percentage was 0.193. These parallelisms strongly suggest that there is a constant relation between the total amount of creatine in the body (or, what for all practical purposes is the same, in the muscles) and the amount of creatinine in the daily urine. Direct observations upon a series of 12 rabbits showed a total body creatine ranging from 4.317 to 0.947 grams with a creatinine output of 99.9 to 19.2 mgm., while the ratio of the two lay between the comparatively narrow limits of 43.2 and 53.3 —in five cases out of the twelve between 44.4 and 45. It would appear therefore that animals with a high creatinine elimination, whatever may be their creatinine coefficient, do actually have a correspondingly high body content of creatine. All these correlations Myers and Fine interpret as indicating that urinary creatinine and muscle creatine are intimately related in metabolism, that the former takes origin either from the latter or from some common precursor, and that the relative constancy of muscle creatine in the normal animal affords an immediate explanation of the uniform rate at which creatinine is eliminated in the urine.

There are many flaws in this argument, which has been severely criticised by Benedict and Osterberg (28); but the case which Myers and Fine make out is none the less a rather strong one. The apparent demonstration of a relation, quite independent of body weight and creatinine coefficient, between urinary creatinine and total body creatine is especially to the point. The thesis has the merit of simplicity, and there are no known facts utterly incompatible with it. It has apparently been adopted by Shaffer (184) as consistent with, and complementary to, his own theory. It leaves of course unexplained the cause and meaning of the relative constancy, in individual and species, of muscle creatine.

Since all but 2 per cent or so of the body's creatine is in the muscles, it is an obvious corollary of the above theory that, so long as the muscles are in a normal state (i.e., possess a normal creatine content), urinary creatinine is determined by muscle mass, and that conversely, if once we ascertained for any species the factor connecting them, the mass of an animal's muscles could be calculated from its creatinine output. This deduction was made by Myers and Fine themselves, but, wisely perhaps, they attempted no practical application of it. Bürger (32) has recently ventured a calculation which would indicate that 1 gram of urinary creatinine per day corresponds in man to 22.9 kgm. of normal muscle. He is on surer ground when he points out that, if creatinine is an index of muscle creatine, the creatinine coefficient becomes under physiological conditions an index of, and indeed proportional to, the participation of the muscles in the body weight. The fact that clinically, as he shows, the coefficient, in the absence of disturbing factors, does vary in a regular way with the apparent predominance of the muscles, lends a certain amount of support to the theory.

ORIGIN OF CREATININE. It was impossible to discuss the metabolic significance of the urinary creatinine without touching upon its origin; but in some respects this may be treated as an independent problem.

It was at one time believed that creatine was readily and largely transformed in the body into creatinine, and the direct derivation of the latter from the former was therefore practically taken for granted. When Folin in 1906 (64) undertook, by the aid of his newly developed colorimetric method, a study of the fate of ingested creatine, these beliefs were for the first time seriously disturbed. The essential data of three of Folin's experiments are reproduced in table 2. (In this table all reported figures for creatine have been converted into terms of the anhydrous substance as such, and a calculation has been made of the amount not accounted for in the urine as either creatine or creatinine; this is reported under the heading of "creatine retained.") The results, as illustrated, may be stated thus: *a*, creatine administration led to no increase of creatinine output; *b*, with doses of about 5 grams a certain amount of creatine was usually excreted unaltered, the proportion so eliminated being greater upon a high than a low protein diet; *c*, with small doses (1 gram or thereabout) no creatine at all reappeared; *d*, in no case was there any increase in urea or, it may be said, in ammonia, or (except once) in the undetermined nitrogen of the urine.

Such results, entirely at variance with prevailing notions, led Folin to conclude that the organism does not possess the power of converting creatine into creatinine, that these two substances are quite independent of each other in metabolism, and that creatine is not, like creatinine, a waste product, but a food.

The observation that creatine introduced from without may be partly excreted unchanged, but is usually to a large extent or even altogether retained within the organism, has been repeatedly confirmed, for animals as well as men, and not only for oral (109), (94), (216), (122), (161a), (72), (202), (118), (176), (144), (172), (74), (76), but for parenteral administration (120), (160), (202), (143), (176), (126). That none of it is ever converted into creatinine has been shown to be an error (94), (160), (202), (72), (143), (176), (144), (172), (126). If the dose given is large enough, the increased output of creatinine is unmistakable. This is strikingly shown by two experiments of Rose and Dimmitt (172), one of which is reproduced in table 2; it will be seen that in this particular case even 1 gram of creatine produced a slight increase of creatinine, while with 20 grams the output rose to 36 per cent above the endogenous level. It may be said that in the other experiment of Rose and Dimmitt, where urea determinations were made, there was no evidence, even after 20 grams of creatine had been given, that any of it was converted in the body into urea or ammonia. On this point Folin's conclusion is fully substantiated. Creatine therefore is not subject in metabolism to any catabolic "destruction," other than by conversion into creatinine.

TABLE 2

Fate of ingested creatine

CREATINE GIVEN	TOTAL N	UREA N	CREATININE	EXTRA CREATININE	CREATINE EXCRETED	CREATINE RETAINED	AUTHOR
grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	
	5.49	4.18	1.33				
0.97	5.39	3.91	1.33	0	0	0.97	Folin
	3.99	2.80	1.34				
	3.80	2.39	1.83				
4.4 (1 dose)	3.67	1.83	1.69	0	0.84	3.56	Folin
	3.76	2.30	1.80				
	19.88	16.44	1.53				
4.4 (3 doses)	21.70	17.69	1.53	0	2.34	2.06	Folin
	20.16	16.96	1.37				
	11.05		1.35				
	10.92		1.29				
	10.42		1.35				
1	10.41		1.45	0.10	0.09	0.79	
2	10.58		1.51	0.16	0.13	1.68	
5	11.48		1.59	0.24	1.81	2.91	Rose and Dimmitt
10	12.83		1.61	0.26	5.81	3.88	
10	13.78		1.69	0.34	5.90	3.70	
20	16.86		1.83	0.48	15.10	4.34	
	11.64		1.67	0.32	0.41		
	9.87		1.61	0.26			
	11.07		1.61	0.26			
	10.40		1.52	0.17			

The demonstration that exogenous creatine is partly transformed into creatinine may not by itself prove that endogenous creatinine has its origin in body creatine, but it disposes of the only experimental evidence irreconcilable with such an idea. The extent of the transformation is admittedly small—roughly 5 per cent of the ingested creatine; but it may be questioned if there is any real reason for expecting it to be greater. The amount of creatinine produced will depend in the first place on the active mass of the creatine at the place where dehydration occurs. There is no way of telling what that "active mass" is; but let it be assumed that it is constituted by the total creatine of the muscles, that the muscles are the sole seat of the reaction, and that accordingly only that portion of the ingested creatine which is incorporated in the muscle substance can contribute to the formation of creatinine. When creatine is introduced from without the muscles are offered an excess of a special tissue component, of which they already as a rule have a sufficient supply; small doses they may be able to absorb completely, but of a large dose much will be rejected, will on the assumption made never enter the metabolic circle, and will accordingly be excreted unchanged. Now the total creatine of the muscles amounts, for an average man, to about 112 grams (32). If that quantity be increased by the addition of 1 gram of ingested and retained creatine, it need not surprise us that the effect upon the creatinine output is imperceptible. In the second of Rose and Dimmitt's experiments (not reproduced in the table) the amount retained out of 20 grams was only 5.7, which is the largest retention ever recorded for a single day's experiment. If all of this were added to 112 grams it would mean an increase of about 5 per cent. The increase of urinary creatinine actually observed was about 0.3 gram; small as it is, it represents an excess of 18.5 per cent over the endogenous output, and is therefore more than one is forced to expect. The argument, as stated, involves several unverifiable assumptions, and in a quantitative sense deals with the crudest approximations; but in its main outline it is probably perfectly sound.

The opinion that creatinine is derived from muscle creatine is supported by the observation (184), (145), (70) that muscle contains more free creatinine than any other tissue, and more than the blood which passes through it (see table 1); as well as by the accelerated and probably enzymatic transformation of creatine into creatinine, without loss of total creatinine, which takes place in muscles or muscle extracts after death (147), (84). The first of these points has its force rather weakened by recent evidence tending to show that blood contains no creatinine at all (20), and the second by the fact that the muscles of birds, which are said to excrete creatine in place of creatinine, show the same changes upon autolysis as those of mammals (147). It may be admitted, therefore, that no single point in the evidence, not even the partial transformation in the body of creatine into creatinine, demonstrates in a clear cut fashion the origin in normal metabolism of creatinine from creatine. Yet, when the entire body of evidence, including the apparent relations between muscle mass or muscle creatine and urinary creatinine or creatinine coefficient, is passed in review, and when it is considered that the actual ability of the body to convert ingested creatine into creatinine is no longer in doubt, it is easy to understand why the old doctrine of the origin of creatinine from creatine meets again today with practically universal acceptance.

One qualification, however, is usually present in the minds of those who subscribe to that doctrine (140), (184). The "creatine" of the living muscles may not be free creatine. There is indeed some difficulty in believing that it is free, for it is present in a concentration very much higher than that in the blood and yet the muscles are able to take up still more creatine from the circulation (70). It would seem therefore that the creatine must be held in the muscles by some special attractive force, either chemical or physical. This leads to the idea of a "creatine-containing complex," and it is possibly some such complex, rather than creatine itself, which is to be regarded as the actual precursor of creatinine. Whether in metabolism this creatine-complex, if it exists, is to be thought of as first yielding creatine, which thereupon is transformed into creatinine, or whether it yields the latter directly, is not very clear.

The second of these alternatives corresponds with the hypothesis of Folin and Denis (70), who still maintain the essential independence of creatine and creatinine; but in their view the creatine-complex is nothing short of the living protoplasm itself. The creatine of muscle, they think, is entirely a post-mortem product. The metabolism of living protoplasm gives rise directly and solely to creatinine; but at death this same protoplasm, in muscle at least, breaks down in such a way as now to liberate creatine. The chief difficulty in accepting this hypothesis is that it implies that the "death" of protoplasm is practically an instantaneous phenomenon; for no matter how suddenly an animal is killed, how rapidly or at how low a temperature its muscles are submitted to extraction, one obtains practically the maximum yield of creatine (158)

If the creatine of living muscles is in some sort of combination it must be a combination of the loosest possible kind. The case is not very different from that of the amino acids. These also accumulate in the tissues from lower concentrations in the blood (210); but it has not been suggested that they therefore become an integral part of the protoplasm, or that they are not themselves the direct precursors of urea. Although doubtless held by the tissues in some kind of loose molecular combination the amino acids behave in metabolism as if perfectly free. Probably the creatine of muscle may be regarded as doing the same.

THE INFLUENCE OF MUSCULAR WORK UPON CREATINE-CREATININE METABOLISM. The *total* output of creatinine for the day is independent not only of the quantity of protein in the diet, but also of the amount of ordinary muscular work performed (93), (181). This appears to be true even when the diet is of inadequate energy value (93). Whether it holds during a complete fast appears to be somewhat uncertain; the recent experiments of Schulz (177) throw some doubt on the conclusion drawn (from a single experiment) by Hoogenhuyze and Verploegh (93) that exercise during fasting causes a considerable rise in the daily excretion of creatinine.

It does not follow that muscular contractions have no effect at all upon creatine metabolism. An effect becomes obvious enough when the output of creatinine is measured not simply day by day, but at intervals as short as two hours. It is then found (177) that the output of any work period is decidedly and invariably greater than that of the corresponding period of a day of inactivity; and that some later period (almost always the one immediately following) exhibits with equal regularity an output unusually low. These opposing effects, which are not noticeably modified by fasting, compensate one another so closely that the net effect upon the output of 24 hours is negligible.

The temporarily increased creatinine excretion associated with work might be the consequence of an increased production of creatine within the contracting muscle, or of an accelerated transformation of the creatine already there, or of both these factors operating together. The choice between these possibilities would obviously be easier if we could learn by direct observation of the muscle itself what changes in its creatine content take place during contraction. Unfortunately no unanimity of opinion upon this point has yet been reached. It has been reported that stimulation of isolated frog muscle causes an increase of total creatinine (31); but also that it produces no effect (159), (175).

Stimulation of muscles in situ with the circulation of the blood intact has been said to bring about a decrease in the frog and in the rabbit (31); but the existence of any effect whatever under such conditions has been denied for the frog (175) and the cat (199). On the other hand cats' muscles stimulated in place after ligature of the main artery of the limb have been found to lose on the average 6.3 per cent of their creatine (199). If all the experimental data merit equal confidence, such divergence of result can have but one meaning—that in the experiments hitherto undertaken some controlling or modifying conditions have been overlooked. One cannot but be reminded in this connection of the perplexities that formerly beset the problem of the production of lactic acid in muscle; and it seems evident enough that the behaviour of muscle creatine during contraction will remain obscure, until it has been studied under conditions as precisely defined and as fully controlled as were those affecting lactic acid by Fletcher and Hopkins. It would be idle to speculate upon the facts as at present imperfectly known. One quite general deduction seems to be all that is justified; that the creatine of muscle is in *some* way, directly or indirectly, affected by muscular work. When the precise nature of the relation has been elucidated, we may be able to explain the temporary effects of work upon urinary creatinine.

THE FATE OF RETAINED CREATINE AND ITS BEARING ON CREATINE METABOLISM. We have seen that when creatine is introduced from without a certain amount is always retained in the body. The quantity which may be so retained is apparently rather limited, since even when presented with as much as 20 grams the human organism has not been observed to keep more than 5.7 grams (172). It is of interest to consider what may become of this.

Since it is certainly not destroyed, two possibilities only seem to present themselves. Either it may be deposited unchanged in the tissues, especially in the muscles, or it may be utilized in the synthesis of other substances. There is no doubt at all that the first of these alternatives can be realized. Folin and Denis (70) observed rapid increases in the muscle creatine of cats who were absorbing creatine from the intestine; in one of their experiments the creatine content of the muscle rose 26 per cent. Myers and Fine (143) were able in this way to account for from 22 to 100 per cent of the creatine retained by rabbits during continued subcutaneous administration; from which it would appear even that the *whole* of the retained creatine may sometimes be simply deposited in the muscles.

If creatinine is an index of muscle creatine, an increase of the latter brought about by the retention of ingested creatine ought to be reflected by a proportional increase in the output of creatinine, not only during the administration of the creatine, but for some time thereafter. Such a continued elimination of extra creatinine is not infrequently to be detected in the records of experiments. A striking instance is seen in the experiment of Rose and Dimmitt (172), as shown in table 2; on the fourth day after the last dose of creatine the creatinine was still decidedly above its normal level. The converse of this effect is possibly represented in the gradual fall of creatinine output which Ringer and Raiziss (166) observed to follow the complete withdrawal of creatine from the diet.

During the six days of creatine feeding in the experiment of Rose and Dimmitt the total amount of creatine retained was over 17 grams. Is it possible that this was simply added as such to the store of creatine already present in the body? As the subject weighed 54 kgm. his muscles probably contained 90 grams; 17 grams more would mean an increase of 19 per cent. The experiments of Folin and Denis, just quoted, show that as a temporary effect such an increase is possible; as a semi-permanent one it hardly seems likely. One is almost forced to assume that some of the creatine was utilized in synthetic processes. The same conclusion is indicated by the figures quoted from Myers and Fine, showing that even when the gain in muscle creatine is allowed for, as much as 78 per cent of the creatine retained may have apparently disappeared.

The evidence therefore suggests that the second alternative also is sometimes realized, that creatine may be of use in the anabolic processes of the body; may, as Folin (64) put it in 1906, serve as a food. In what manner it does so can only be guessed. Its chemical relationship to choline points to a possible use in the synthesis of lecithine; by its guanidine radicle it might be supposed to take part in the formation of arginine (158). If Cathcart (37) is right in holding that carbohydrate is necessary to its utilization, while fat is of no service, the latter supposition would be the more probable.

If creatine does function as an anabolite, its utilization is almost certainly accomplished entirely in the muscles. For in muscular atrophies or dystrophies 90 per cent or more of administered creatine, even of small doses, appears unchanged in the urine (122), (76).

Creatinuria. While the urine of the adult male on a creatine-free diet contains no creatine, that substance is a normal and constant associate of creatinine in the urine of children of either sex up to the age of puberty. In women also creatinuria is a physiological occurrence although only an intermittent one. Both in men and in women creatinuria may be induced or increased by a great variety of experimental or pathological conditions. The lower mammals behave in these respects very much like the human species, but exhibit still more readily both the spontaneous and the induced varieties of creatinuria. Ruminants (cattle, sheep and goats), for example, regularly excrete considerable quantities of creatine (153).

The most important forms of induced creatinuria are those produced by starvation, carbohydrate deprivation (including diabetes mellitus, pancreatic diabetes, and poisoning by phlorhizin, hydrazine (127), (205), adrenalin (204) or sodium selenite (41)), wasting diseases generally, exophthalmic goiter (182), (48), fever (109), (182), (183), muscular atrophies and dystrophies (122), (76) and perhaps acidosis. As it would be impossible to consider every one of these conditions, discussion will be limited to those bearing most directly upon the physiological problem.

Creatinuria of starvation and carbohydrate deprivation. The fact that complete fasting usually brings about an excretion of creatine was first observed almost simultaneously by Cathcart (37) and F. G. Benedict (22), (23) in man, and by Dorner (55) in the rabbit. Many others have since confirmed the observation for these species (98), (26), (135), (141) or extended it to others like the dog (205), (163), (97), (99), (100), sheep (101), pig (188) and guinea pig (155). In many of the reported observations, the disturbing effect of the concomitant ketonuria upon the analysis (78), (77), (29) has been unrecognized or disregarded, but the creatinuria is nevertheless a real one (40), (138), and the only uncertainty with which the data are sometimes affected is a quantitative one.

Different individuals and different species vary in the rapidity with which fasting creatinuria sets in, and in the intensity which it assumes. In the pig it is sometimes impossible to produce a starvation creatinuria at all (130), (188). Even the dog may at certain stages of a prolonged fast excrete no creatine (100). Such differences depend no doubt upon differences in the nutritive condition of the animal at the commencement of the fast (135), or upon its relative ability to utilize fat in shielding the tissues from disintegration (130).

The creatine output during starvation bears no constant relation to either the total nitrogen or the creatinine nitrogen. Sometimes there is observed a parallelism or even a strict proportionality between total nitrogen and "total" creatinine (135), (141), and sometimes the latter maintains an almost constant value throughout the fast, as if to suggest that fasting merely diminishes the proportion of waste creatine transformed into creatinine (22); but neither of these phenomena is a regular occurrence (218).

Starvation involves the consumption by the organism of its own protein, probably in the first place chiefly the "reserve" or "circulating" protein, but as this diminishes an increasing proportion also of the "tissue" or fully organized protein. The latter must be furnished largely by the muscles, which lose 42 per cent of their weight in a dog starved for twenty-four days. As the muscle disintegrates, its creatine, it is reasonable to suppose, will be liberated. Such internally liberated creatine might be expected to behave just like creatine introduced from without; in which case, since the starving organism is living on a fairly high protein level, some of it would be retained, but some excreted unaltered. The retained creatine would in part perhaps be utilized, in part simply deposited in the remaining muscular mass, the creatine content of which is actually found to be raised during the earlier part of a fast (136), (141). In the later stages of starvation creatine for some reason, totally unexplained as yet, is lost even from that muscle tissue which remains apparently intact (97), (141); this might explain why the creatine output rises disproportionately towards the end of the fast (100).

The direct derivation of urinary creatine from the preformed creatine of muscle was suggested by F. G. Benedict (22), was admitted by Mendel and Rose (135) as a contributing factor in hunger creatinuria, and was definitely adopted by Myers and Fine (141) as a complete explanation of that phenomenon. This view has an obvious simplicity in its favour which would make one loath to abandon it until it has been shown to be incompatible with the facts. It is supported by such an observation as that of Frontali (73) who found that the marked creatinuria which follows total thyroidectomy in dogs is accompanied by (and probably due to) a very considerable loss of creatine from the muscles. It is not invalidated by the fact that the amount of muscle disintegrated, as calculated from the output of creatine, does not often agree with the amount calculated from the total nitrogen loss. As a matter of fact muscle loss cannot be calculated from either of these data. The nitrogen of the urine is in part derived from sources, such as reserve protein or organ protein like that of liver, which yield little or no creatine, a factor which would make the proportion of creatine to total nitrogen lower in urine than in muscle, as is usually the case. On the other hand the protein nitrogen of the disintegrated muscle may be only in part excreted, in part utilized in more essential tissues by synthetic processes which do not involve the simultaneously liberated creatine; in such circumstances the creatine output would tend to outstrip the total nitrogen, as it sometimes actually does (99). Paton (156) in 1910 not only pointed out these theoretical possibilities but attempted to illustrate them by experiments upon ducks fasted after different types of feeding and starting therefore with different amounts of surplus protein. Still other factors disturbing the relation between total nitrogen and creatine are the possible retention of muscle creatine, suggested above, and the selective loss of creatine from muscle tissue which seems to take place in the terminal stages of a fast.

Before weighing certain objections that have nevertheless been taken to Myers' and Fine's explanation of starvation creatinuria, it is necessary to consider some other aspects of the phenomenon.

In 1909 Cathcart (38) showed that the creatinuria of starvation was promptly abolished in man by the administration of a practically protein-free carbohydrate diet, but not (as he thought) by the administration of fat with protein. The effect of carbohydrate was confirmed by Mendel and Rose (135) for rabbits, and by Wolf and Osterberg (218) for dogs. Cathcart offered, to explain his results, the hypothesis that creatine is produced in the organism in considerable amounts, but is normally utilized in synthetic processes for the accomplishment of which carbohydrate is indispensable; in the absence of sufficient carbohydrate, as in starvation, the creatine, which cannot now be utilized, is excreted. Mendel and Rose, who like Cathcart found protein and fat to be ineffective, agreed that carbohydrate is specifically essential to normal creatine metabolism, but suggested that it might be concerned in the transformation of creatine into creatinine. It presently appeared, though, that the effect of carbohydrate in preventing inanition creatinuria was not so specific as had been thought. Graham and Poulton (77) showed that mere deprivation of carbohydrate, as brought about for a few days at least by a diet of fat or fat with protein, does not lead to an excretion of creatine in man; the creatine observed by Cathcart upon such diets being shown to be probably an analytical error due to ketonuria. Wolf and Osterberg (218) had already shown that in dogs serum protein will abolish the creatine of starvation almost as effectively as starch; and Rose, Dimmitt and Cheatham (171) proved that a diet of eggs (protein with fat) will do the same for man. In the pig a pure fat diet, even if continued for a long period, does not necessarily lead to creatinuria (131), nor does a pure starch diet necessarily prevent it (188). The effect of carbohydrate therefore is neither unique nor certain. It is possible that it is, as Myers and Fine suggest (142), merely one phase of the well known sparing action of carbohydrate upon protein catabolism. The apparent impossibility of abolishing starvation creatinuria by fat alone (218) may be due partly to its relative inefficacy as a sparer of protein, partly to the acidosis which it induces, partly to the practical difficulty of feeding enough.

There are, nevertheless, certain other facts which are believed to speak for a special connection between creatine metabolism and carbohydrate. If the body tissues are deprived of carbohydrate by phlorhizin (39), (114), (115), (217), (135), (28), or lose the ability to utilize carbohydrate as in diabetes mellitus (14), (115), (191) or pancreatic diabetes (170), creatine is invariably excreted. The amounts found appear to bear an intimate relation to the extent to which carbohydrate is actually withdrawn from the tissues; in phlorhizinized starving dogs they become relatively enormous. Of course the conditions named involve not merely withdrawal of carbohydrate, but also as a rule a concomitantly increased catabolism of protein, and, as far as the data permit one to judge, the creatinuria seems under most conditions to run parallel with the latter (39), (217). S. R. Benedict and Osterberg (28) claim to have shown that it is none the less entirely independent of tissue destruction; and, as their observations constitute by far the most formidable difficulty in the way of identifying the urinary creatine of starvation or carbohydrate deficiency with preformed creatine set free from disintegrated or altered muscular tissue, they must be carefully considered.

Benedict and Osterberg found that it was possible to feed to fasting phlorhizinized dogs such amounts of creatine-free protein as would nearly or altogether abolish the negative nitrogen balance. They argue thereupon that "if the creatine of the urine has its origin in the destruction of muscular tissue . . . . , a sparing of the body tissue destroyed by feeding exogenous protein should cause a corresponding fall in the creatine eliminated, whereas if the utilization (or destruction) of creatine be dependent upon carbohydrate utilization, the ingestion of exogenous protein in the phlorhizinized dog should not appreciably affect the output of creatine in the urine." The experimental results are held to settle quite definitely the choice between these alternatives. The creatine output, it is claimed, reveals itself as totally independent of the state of the nitrogen balance. "Creatine may be eliminated in the urine in large amounts without any corresponding loss of body tissue." The creatine excreted cannot therefore simply represent preformed muscle creatine liberated by the dissolution of flesh. Creatine, it is concluded, must be constantly "formed in the animal organism in relatively large amounts," but "is normally for the most part either utilized or destroyed." This disposition of the creatine is dependent upon the utilization of carbohydrate, and when that is impossible, as in phlorhizinized animals, the constant production of creatine is revealed by its elimination. The success of protein in preventing creatinuria in normal dogs is attributed to its capacity for the formation of glucose; its failure in phlorhizin poisoning to the withdrawal of even proteinogenous glucose from the tissues.

It is doubtful if Benedict and Osterberg's data furnish adequate proof of these propositions. They demonstrate at the utmost nothing more than the production, independently of tissue catabolism, of notable amounts of creatine *in phlorhizinized animals.* To infer a similar extensive production in normal animals is hardly justifiable. The production in phlorhizin poisoning of excessive amounts of sugar from protein does not prove that the metabolism of amino acids in the normal organism necessarily involves the intermediate production of glucose. To show, by a drastic interference with normal metabolism, that the dog possesses the capacity to form more creatine than it can utilize, does not prove that under physiological conditions it exerts that capacity any further than the need of the organism for creatine may require. The results of Benedict and Osterberg are striking and important, and must be seriously reckoned with; but to the writer they seem to leave still open the questions of the relation between starvation creatinuria and muscle waste, and of the unique importance of carbohydrate in creatine metabolism. Their confirmation, upon pancreatectomized dogs, by Rose (170) adds of course nothing to their real significance.

It may be added that Cathcart, if the writer understands him correctly, has receded somewhat from his earlier views, and now regards the output of creatine in conditions of carbohydrate deficiency not so much as evidence of the disturbance of creatine metabolism *per se,* but rather "as an index of faulty metabolism in general" (41), (42); and he seems inclined to relate it, sometimes at least, directly to the creatine content of the muscle tissue catabolized (41).

The creatinuria of high protein feeding. In 1912 Folin and Denis (65) suggested as an explanation of the creatinuria of children that it is due to an excessively high level of protein consumption in proportion to muscular mass; but McCollum and Steenbock (130) appear to have been the first to definitely postulate an *exogenous* as well as an endogenous origin of urinary creatine, and to produce experimental evidence in its support. They came to the conclusion that not simply the plane of protein intake, but the character of the proteins in the diet, determines the extent of creatine production. They found, for example, that an abundantly fed pig receiving five times its endogenous protein requirement in the form of linseed and gluten meals excreted regularly considerable quantities of creatine, whereas if it received the same quantity of protein or even twice as much from corn alone the urine seldom contained any creatine at all. Steenbock and Gross (188) have more recently shown that in fasting pigs the administration of casein in sufficient amount will induce creatinuria when that is absent, or increase it when already present. This is in remarkable contrast to the observed inhibitory effect of serum or egg proteins upon starvation creatinuria in dogs (218) or men (171), and emphasizes the probable importance of the kind of protein fed.

Further evidence for an exogenous origin of urinary creatine has been found by Denis and her associates in the facts, *a*, that the output in a variety of conditions (exophthalmic goiter, childhood, etc.) appears to bear a relation to the meals (always creatine-free) of the subject, being very much smaller at night than during the day, and usually attaining a maximum in about two hours after the substantial meal of the day (47), (49); *b*, that the amount of creatine excreted by cases of exophthalmic goiter (male or female) is increased by high protein feeding and decreased or reduced to zero by a low protein diet (48); *c*, that the creatine output of children is increased by a diet rich in protein, and diminishes or even disappears upon a minimum protein intake (49); *d*, that creatinuria can be produced in adult women by forced protein feeding and made to disappear again by lowering the protein intake (50), (52). Gibson and Martin (76), again, found that the creatine output in pseudohypertrophic muscular dystrophy is intimately related to the protein intake, their data being of particular interest because in the condition named ingested creatine is excreted quantitatively, so that their subject was practically "diabetic" as regards creatine. They made the further very important observation that it is only the exogenous protein immediately catabolized, and not that retained for growth that affects the creatine output.

The view that creatine may, in part at least, have an exogenous origin was supported by Harding and Young (85) on the basis of data (as yet unpublished) obtained from growing puppies. On the other hand Powis and Raper (162), who first observed the periodic variations in the creatine output of children, found these quite unrelated to meals; Rose, Dimmitt and Bartlett (173) were unable to induce creatinuria in women by high protein feeding; and in the case of normal men it has not been found possible to bring about on the highest attainable plane of protein intake (even with 33 to 35 grams of nitrogen in the urine) any excretion of creatine whatever (50). Such failures do not destroy the significance of positive observations; but they emphasize the difficulty of producing creatinuria by protein feeding alone.

It is of course an apparent inconsistency that protein may sometimes, as when fed to starving dogs or men, abolish an existing creatinuria, and sometimes, as in the experiments of Denis and her collaborators, induce one where it was absent. If it is true that only that protein is effective which is catabolized, the inconsistency becomes less glaring; for much of the protein fed after a period of starvation may be simply retained in the body, as was certainly the case with the dogs of Wolf and Osterberg (218). This conception may also explain why differently constituted proteins should vary in their effect. The data for the experiments of Denis and Minot upon women (50) do not permit us to calculate exactly the nitrogen balance, but as their diets contained a large amount (50 grams) of gelatin, it is probable that the proportion of food nitrogen promptly excreted by their cases was relatively high. This may account for the difference between their results and those of Rose, Dimmitt and Bartlett.

Although an effect of food protein upon creatine production appears to have been fully demonstrated, this is not necessarily to be interpreted as proving an exogenous source for creatine, in the sense that the latter may arise directly, like urea, from certain precursors in the ingested protein molecule. There may be another explanation of the phenomenon. It may represent merely one phase of that general stimulation of cellular metabolism which is described as the specific dynamic action of protein. An increased *endogenous* production of creatine, resulting from such stimulation, might be expected to manifest itself in just the sort of creatinuria that follows protein ingestion. It would be brought about only by catabolized protein, since protein deposited in the form of new tissue exerts no specific dynamic action (125); it would be at its height, as Denis and Kramer found it to be (49), during the second and third hours after ingestion, when heat production has reached its maximum but the output of urea is still rising (212); and it would occur most readily in those conditions where the metabolism is already relatively high, as in exophthalmic goiter, or in childhood. Such an explanation of the creatinuria following a high protein diet seems therefore to be well worthy of consideration. Protein feeding will increase also the output of uric acid; but no one has sought the origin of uric acid directly in the protein of the diet. Lewis, Dunn and Doisy (123) have shown reason to believe that proteins and amino-acids increase the production of endogenous uric acid by virtue of their general property of stimulating all cellular metabolism; it does not seem improbable that they should simultaneously increase the production of endogenous creatine.

Creatinuria in children. The fact that normal infants and children of both sexes usually excrete creatine as well as creatinine was discovered by Rose (168), confirmed by Folin and Denis (65) and by Krause (118), and has formed the subject of further study by Powis and Raper (162), Denis and Kramer (49) and Gamble and Goldschmidt (74). The creatinuria in question is of course a physiological phenomenon, and must depend upon quantitative rather than upon profound qualitative differences between child and adult. Probably it represents a temporary survival of an earlier stage in the evolution of the type of creatine metabolism which now characterizes adult man.

The precise point, or points, in the metabolic cycle, at which children differ from adult men, is not quite clear. It might be that children produce relatively more creatine; or that they produce, relatively to their muscle mass, the same quantity of creatine as adults, but have a less completely developed capacity to retain (or assimilate) it. Actually the creatine content of the immature muscle is lower than that of the fully developed (see table 1), as if it were more readily "saturated" with creatine; and in accordance with this conception children are found to excrete a relatively large proportion of ingested creatine (118), (162)—infants as much as 100 per cent (74)—just as do the subjects of muscular dystrophy (122), (76). The avidity (if it may be so expressed) of the muscle for creatine and its efficiency as a machine develop together, and as they develope creatine disappears from the urine. It is also possible that children have a relatively low power to convert muscle creatine into creatinine. On this point we have as yet no very direct evidence.

Creatinuria in women. In contrast to normal men, normal women even upon a creatine-free diet excrete, although only intermittently, small quantities of creatine. This was first observed by Krause and Cramer (116), (117), who thought that the creatinuria had a definite relation to the menstrual period. Others (167), (173), (187) have been unable to observe any regularity whatever in the output.

It appears not unreasonable to suppose that the creatinuria of women represents merely an imperfect transition from the creatine metabolism of childhood to that of vigorous adult life, and that it is associated with the relatively poor muscular development and low creatinine coefficient of the female sex in general. This would imply that the female organism has a lower power of assimilating creatine than the male. The experiments of Stearns and Lewis (187) upon the fate of ingested creatine in women seem to show that this is not the case. It is to be noted, though, that the two subjects they studied did not excrete spontaneously more than a trace of creatine, and that indeed their urine was often creatine-free for weeks together. In other words these women, in regard to creatine metabolism, gave hardly any indication that they differed from men. Perhaps, if the point were examined, it would be found, as Stearns and Lewis seem to infer, that women leading a life of abundant muscular activity exhibit no creatinuria at all.

The generally intermittent creatinuria of women becomes continuous during pregnancy (114), (117), (95), increasing toward the end so that in the last few weeks before parturition it may average 0.17 gram daily (211). This may be correlated in some way with the fact that the uterus during pregnancy acquires both absolutely and relatively more creatine that the resting organ (21). Immediately after delivery the output of creatine rises further still, sometimes reaching the extraordinary figure of 1.5 grams in 24 hours (182), and averaging 0.42 gram daily in the first four days of the puerperium (211). According to Heynemann (90), this post-partum creatinuria is at its height, in the human subject, on the third and seventh days; in the dog Murlin (139) found a maximum on the fifth day. Shaffer (182) and Murlin (139) attributed the phenomenon to the rapid escape of creatine from the involuting uterus. This explanation is extremely plausible, and one can hardly doubt that it correctly states one factor at least; but it does not seem to be certain that the gravid uterus contains enough creatine to account for the total quantity excreted. Beker (21) calculates that the human uterus in returning to the non-pregnant condition loses 0.73 gram of creatine; the total excess output of the puerperium must often be greater than this. Mellanby (134) brings the post-partum creatinuria rather into relation with the onset of lactation. The excretion of creatine during pregnancy and the puerperium is in obvious need of further and more detailed study.

Creatinuria and acidosis. A great many of the conditions resulting in creatinuria—starvation, carbohydrate deficiency, diabetes, fever, etc.—are characterized by an abnormal production of aceto-acetic acid or other acid substances, and therefore by a tendency to depletion of the alkaline reserve, or acidosis. This suggested to Underhill (206) in 1916 the hypothesis "that a condition of acidosis in the body is responsible for the appearance of creatine in the urine." After testing this hypothesis in a variety of ways upon rabbits (207), (208), (209), he concluded that there is indeed an interrelationship between acidosis and creatine elimination, but that neither acidosis nor carbohydrate deficiency can be the sole factor in the production of every type of creatinuria. A similar conclusion is reached by Steenbock and Gross (188) on the basis of experiments upon pigs. Denis and Minot (52), on the other hand, could observe no definite effect upon creatine output on administering sodium bicarbonate to boys or women. Sawyer, Stevens and Baumann (174) produced in children a demonstrated reduction of alkaline reserve by giving a high-fat low-carbohydrate diet; the creatine output was always increased, but the authors themselves do not believe that the acidosis in itself was the responsible factor. Gamble and Goldschmidt (74) were also unable to secure, by adding acid or base to the food of infants, any evidence of a relation between creatinuria and the acid-base balance of the diet.

It is quite certain that acidosis is not in any condition at present known the sole determining cause of creatinuria, and it is doubtful if it is often an important contributing one. That it may sometimes play a subsidiary part is not impossible, and may even, in view of its stimulating effect upon cellular metabolism, be considered likely.

Upon a general review of the whole situation with regard to creatinuria it appears very improbable that the condition can be referred in all of its types to any single cause. Probably creatinuria is always endogenous in origin and most examples, if not all, could be included under one or more of the following descriptions:

1. Creatinuria due to dissolution of muscular tissue; as in starvation, carbohydrate deficiency, etc.

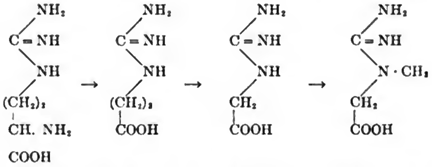
2. Creatinuria due to over-stimulation of endogenous metabolism; as in fevers, exophthalmic goiter, high-protein feeding, etc.

3. Creatinuria due to defective power to store, or utilize, or dehydrate creatine produced in normal amounts; as in childhood, muscular dystrophies, etc.

ORIGIN OF CREATINE. It is generally assumed, and the assumption has every element of probability in its favor, that creatine is derived from protein. Of the mechanism of its origin and of the nature of its immediate precursors we possess no certain knowledge. None of the supposed precursors which have hitherto formed material for experiment has been shown to satisfy the two requirements necessary to establish it in the status of an actual intermediate: *a*, that it should, upon administration in the proper way (that is, in such a way as to reach the actual locus of transformation), be converted readily and abundantly into creatine (or creatinine); and *b*, that it should be detectable in traces at least as a normal constituent of the body or of the urine. If creatine were a waste product the almost uniform failure to connect it with hypothetical precursors would almost prove that the real precursor has not yet been thought of. As we have seen, creatine is probably not a waste product, but either an integral part of the living protoplasm (70), or a tissue constituent with a special function. Its rate of production is therefore in all likelihood regulated by the internal demand, and it is not to be expected that it should be accelerated by an excessive supply of precursors, any more than the production of adrenalin or thyroxin would be increased by the administration of large doses of tryptophane. One encounters rather frequently, it is true, the idea that creatine is constantly being produced in relatively large quantities, and as constantly in some mysterious way being "destroyed." The experimental work reviewed in the preceding pages affords no proof of, and little support for, such a conception. We have positive knowledge of creatine production only in connection with the growth or renewal of protoplasm in certain tissues; and creatine is not "destroyed" in any other way than by conversion into creatinine, although it may possibly be utilized in unknown anabolic phases of metabolism. Folin and Denis (70) explain the failure to trace creatine to any known food constituent by an essentially identical argument.

Speculation and experiment upon the origin of creatine have centered mainly upon arginine, the only known protein constituent possessing a guanidine radicle.

1. Arginine as the mother substance of creatine. On the theory of Knoop (110) and Neubauer (149) creatine arises from arginine by the following series of reactions:


Arginine
γ-guanidino-butyric acid
guanidino-acetic acid (glycocyamine)
methylguanidino-acetic acid (creatine)

Up to the formation of glycocyamine this scheme follows the well established lines of amino-acid catabolism by deamination and β-oxidation. The final step in the process—the methylation of glycocyamine—is one which, it is generally agreed, the animal body has been demonstrated to be capable of taking (106), (55), (154), (197), (16), (76). The scheme has therefore a certain plausibility, increased rather than diminished by the fact that, as Neubauer himself points out, it could not be expected to apply to *ingested* arginine, the guanidine group of which would be converted into urea by the arginase of the liver, but would readily account for the appearance of creatine in the special metabolism of muscle, where arginase is not to be detected.

Attempts to secure direct evidence of the conversion of arginine into creatine have for the most part given admittedly negative or inconclusive results (106), (13), (17), (195). Positive results are reported by Inouyé (104), who obtained small increases of total creatinine upon incubation of arginine with hashed liver, or perfusion through the surviving organ; by Thompson (197) who observed increases of urinary creatine in dogs, birds and rabbits, after injection or oral administration of arginine; and by Gross and Steenbock (81) who confirmed Thompson's results by feeding the base to pigs. The significance of Inouyé's data is doubtful; but as the arginine in his experiments must have been promptly hydrolyzed by the liver arginase, its guanidine group can hardly have been responsible for any observed formation of creatine. In Thompson's experiments the extra creatine never corresponds to more than a small fraction—from 1.1 to 4.5 per cent—of the guanidine nucleus introduced; no control experiments were made with other amino-acids; and it is not at all impossible that what was observed was a general amino-acid effect of stimulation. The data of Gross and Steenbock distinctly support such an explanation; for while they observed increases of urinary creatine upon feeding arginine, they obtained increases greater still from casein of equivalent arginine content. None of these experiments therefore furnish conclusive evidence of a conversion of arginine into creatine. Evidence more direct is possibly to be found in experiments of Jansen (108), who reports that increased tonus of frog's leg muscle is accompanied by a disappearance of arginine and the production of a corresponding amount of creatine.

As might be expected no unequivocal effect upon muscle creatine (146), or upon creatine excretion (86), (76),or creatinine output (93) is produced by exchanging an arginine-poor protein in the diet for an arginine-rich one.

In the endeavor to establish a connection between creatine and arginine, experiments have been made also with many possible intermediates and their higher homologues. Neither γ-guanido-butyric (192) nor ϵ-guanidino-caproic (193) acids, nor the corresponding methylamino or methylguanidine derivatives (194), nor yet δ-methylarginine (195) give any evidence of convertibility into creatine in the animal organism. Among all the conceivable intermediates of an arginine-creatine transformation glycocyamine remains the only one which gives positive results; and there are difficulties in accepting it as a real intermediate of normal metabolism. It is for the most part excreted unchanged, only a fraction of the administered dose undergoing methylation; and although only slightly soluble, it has never been detected as a constituent of animal tissues.

In the face of such almost uniformly negative results one is almost forced to assume that, if creatine is related to arginine at all, its mother substance is not free arginine but the still combined arginine of the muscle protein. Seemann's (180) hypothesis of the existence in protein of a preformed creatinine ring, in the construction of which the guanidine group of arginine is supposed to participate, contains an inherent improbability; for all the evidence indicates that the guanidine groups of the protein molecule are free (113). It is more likely that as Thomas (195) suggests, the projecting arginine side-chains undergo in tissue metabolism β-oxidation from the terminal guanidine group inwards, the disrupted fragment being then converted into creatine. Such a speculation readily falls in line with the view of McCollum (129) that endogenous metabolism does not involve the complete disintegration of the protein molecule. Complete disintegration of the molecule would indeed be difficult to reconcile with an origin of creatine (and creatinine) from arginine alone; for arginine contributes but 11.1 per cent, its guanidine nucleus therefore 8.3 per cent, of the nitrogen of muscle and tissues in general (56), whereas, when the metabolism of protein is reduced to a purely endogenous level, creatinine may form 18.5 per cent of the total nitrogen output (128).

Support for the conception that creatine is derived from arginine has been found in the fact that while the muscles of vertebrates contain creatine but no free arginine, those of invertebrates (crustaceans, insects, molluscs), which contain no creatine, yield larger or smaller quantities of free arginine (2), (4), (6), (7), (119), (152).

2. *Creatine as a detoxicator of guanidine.* Noel Paton (158) has recently revived the hypothesis of Jaffé (106) and Achelis (1) that creatine is the product of a reaction having for its object the detoxication of guanidine or methyl-guanidine. Guanidine is presumed to arise from the complete oxidative decomposition of arginine, from some other unknown guanidine grouping in the protein molecule, or even under certain circumstances—as in the developing chick (34)—from non-guanidine nitrogen. Methyl-guanidine is generally believed to be a normal constituent of flesh (185) and urine (57). Both bases are highly toxic, producing symptoms similar to those of tetany (157). In tetaniaparathyreopriva (112), (33) and in idiopathic tetany (33) their concentration in blood or urine or both is greatly increased. After removal of the parathyroids the creatine content of muscle increases while its total guanidine concentration falls (87). In spite of negative results (chiefly with methylguanidine) by others (106), (55), (164), (1), (17), Thompson (198) found an increase of creatinine output in the dog, and of creatine output in the duck, while Wishart (215) obtained increases of muscle creatine, after injections of guanidine salts. When it is considered that it is by no means certain (59), (18), (80) that the methylguanidine found in flesh and elsewhere is not an artificial product, the evidence hardly seems to form a very strong case for the detoxication theory.

3. *Creatine as a product of methylation.* The derivation of creatine from arginine or from the guanidine nucleus of protein involves at some point or other a process of methylation. Methylation and methylated compounds are specially characteristic of the vegetable kingdom (witness the numerous plant betaines and methylated alkaloids), but are by no means uncommon among animals. Choline (as a constituent of lecithine), adrenalin, carnitine (probably

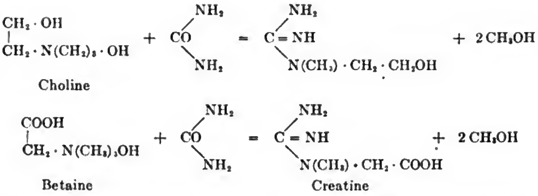
$$(CH_3)_3 \vdots \underline{N \cdot CH_2 \cdot CH(CH_2OH) \cdot CO \cdot O}$$

(58) and glycocoll-betaine are examples of methyl compounds with wide distributions in the animal kingdom. γ-Butyro-betaine is found in the urine of dogs poisoned by phosphorus (190). Pyridine (91), (96), (201), tellurium salts (92) and nicotinic acid (3) given by mouth are excreted as methyl derivatives. The instance of glycocyamine and its conversion to creatine has already been noted.

Neither the purpose nor the mechanism of methylation are perfectly clear. It has been thought that it serves to protect the substance involved from further catabolism. Certainly the betaines and methyl compounds in general are very resistant to oxidation in the animal organism (5), creatine, as we have seen, being no exception to this rule. It is possible then that the formation of creatine serves to preserve for special uses the guanidine group of arginine.

In plants methylation is believed to be effected by the union of formaldehyde with amino groups, and reduction of the methylene-amino compounds thus produced. Thompson (200) has suggested that creatine may arise in a similar way by the action of formaldehyde upon guanidino-acetic acid or some earlier stage in the oxidative catabolism of arginine. He supported his hypothesis by experiments in which the administration, especially the subcutaneous administration, of paraformaldehyde or urotropin with or without arginine to ducks appeared to give notable increases in the output of creatine. It may be objected that formaldehyde is not known to arise in the animal body, that if it did there seems to be no reason why it should couple with guanidine rather than with the far more abundant free amino groups in the tissues, and that Thompson took no account in his analyses of the fact that formaldehyde gives the Jaffé reaction. There seems therefore to be no real evidence that creatine formation represents a mechanism for the detoxication of formaldehyde, or that the latter has anything to do with its origin.

4. *Creatine as a derivative of choline or betaine.* Koch (111), in 1905, suggested that creatine as a methylated amino-acid derivative might be related to the metabolism of lecithine, of which the methylated amino-alcohol choline is a characteristic component. Riesser (164) in 1913 developed this hypothesis in considerable detail, pointing out that not choline alone but also its oxidation product betaine might be supposed to be capable of condensing with urea, according to the following schemes:



$$\begin{array}{l} CH_2\cdot OH \\ | \\ CH_2\cdot N(CH_3)_3\cdot OH \end{array} + CO\begin{array}{l} / NH_2 \\ \backslash NH_2 \end{array} = C\begin{array}{l} / NH_2 \\ = NH \\ \backslash N(CH_3)\cdot CH_2\cdot CH_2OH \end{array} + 2\,CH_3OH$$

Choline

$$\begin{array}{l} COOH \\ | \\ CH_2\cdot N(CH_3)_3OH \end{array} + CO\begin{array}{l} / NH_2 \\ \backslash NH_2 \end{array} = C\begin{array}{l} / NH_2 \\ = NH \\ \backslash N(CH_3)\cdot CH_2\cdot COOH \end{array} + 2\,CH_3OH$$

Betaine

Creatine

Betaine would thus give creatine directly, while the condensation product with choline would yield it upon simple oxidation. On this theory the guanidine group of creatine would be formed synthetically, and not derived from any preformed residue in the protein molecule.

In support of his hypothesis Riesser reports experiments upon rabbits indicating that both the creatine content of muscle (164), and the daily output of creatinine (165) may be increased by subcutaneous injections of choline, or of betaine. Thompson (198), on the other hand, found no very decided effect of either substance upon total urinary creatinine in the dog; and Baumann and Hines (17), who perfused urea with betaine or choline through dog's muscle, obtained no increase of creatine with the former and only a doubtful one with the latter. The experiments of Riesser therefore, although exceedingly suggestive, do not suffice to establish either choline or betaine as precursors of creatine.

It might be pointed out that betaine at least, in the rôle of a creatine precursor, would not have any necessary connection with lipoid metabolism, but as a fully methylated glycocoll might equally well be derived from protein. Indeed it is almost certain (11) that the betaine found in living organisms arises not from choline but from glycocoll. As glycocoll can apparently be produced in almost unlimited quantities in the endogenous metabolism of protein (at the expense of a corresponding quantity of urea) (132), it is unnecessary to look elsewhere for a constant source of betaine in Riesser's hypothetical reaction. Now it is of no little interest that while betaine is absent as a rule from the creatine-containing muscles of vertebrates, it has been rather frequently found in those of invertebrates. It has been detected in the mussel (30) (7), the shrimp (2), the crayfish (119), the octopus (88), the scallop and the periwinkle (213), the spiny lobster and *Loligo* (152), none of which yield even traces of creatine. It would seem to be of particular importance that in some of the lowest vertebrates, for example the lamprey (213) and the dogfish *(Acanthias vulgaris)* (189), creatine and betaine are found together. Such data of comparative biochemistry, scattered and incomplete as they are, certainly suggest that betaine is a step in the evolution, and probably therefore in higher forms in the production, of creatine. The occurrence of betaine in ox-kidney (19), if it should be confirmed, makes its position as a real intermediate still more probable.

Betaine as an intermediate product is of course not incompatible with arginine as the original mother substance of creatine, for arginine accompanies betaine in many invertebrates, and betaine might conceivably take origin from ornithine, through the preliminary stages of γ-amino-butyric acid and glycocoll. Methylation might be the final step, or might occur at either of the earlier stages. Carnitine, a regular constituent of vertebrate muscle, was at one time thought to be the hydroxy-derivative of methylated γ-amino-butyric acid, and as such might have figured as a possible relation of creatine; but it appears to have in reality a different constitution (58).

5. *Creatine as a derivative of cystine.* Harding and Young (86) have suggested that creatine may be derived from cystine "through the intermediate stages of taurine and amino-ethyl alcohol, followed by methylation, combination with urea, and oxidation." They have not yet published the experiments which led them to this hypothesis. Gross and Steenbock (81), who observed increases of creatine in pigs after feeding cystine, attributed their results to acidosis consequent upon the oxidation of the sulphur. Gibson and Martin (76) found cystine to be without effect upon the creatine output in pseudohypertrophic muscular dystrophy. It seems unlikely, in any case, that cystine could be the source of all the creatine produced in the body.

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THE BIOCHEMICAL JOURNAL

EDITED FOR THE BIOCHEMICAL SOCIETY
BY
W. M. BAYLISS, F.R.S., and ARTHUR HARDEN, F.R.S.

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The main purpose of PHYSIOLOGICAL REVIEWS is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Biophysics, Experimental Pharmacology and Experimental Pathology.

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